Development and Characterization of Covalent Inhibitors of the RAS-PIK3CA interaction

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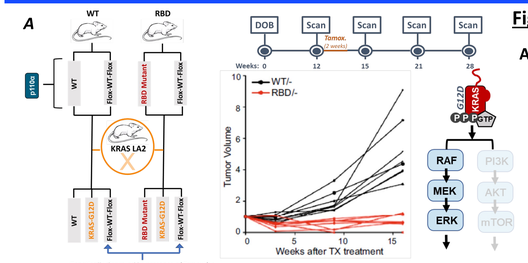
Abstract

RAS proteins are membrane bound GTPases that when hyperactivated, act as oncogenes through activation of the MAPK & PI3K pathways. Each of these pathways has oncogenic potential, with excessive MAPK pathway activation being a common feature of melanoma while aberrant activation of the PI3K pathway is associated with breast cancer . Simultaneous activation of these pathways, as occurs in RAS driven cancers , generates aggressive cancers that present a significant clinical challenge. KRAS, the most commonly mutated RAS isoform, is also the most frequently mutated oncogene in cancer . While treatment options have improved for a subset of these patients due to the accelerated approval of KRAS-G12C inhibitors, the rapid development of resistance highlights the continued need for effective treatments. In RAS driven cell and animal models, dual inhibition of the MAPK & PI3K pathways has shown superior efficacy relative to targeting the individual pathways, however dose limiting toxicities in humans have prevented this combination strategy from finding clinical success.

While physiological activation of the MAPK pathway is RAS dependent, the interaction between RAS and the catalytic subunit of PI3Kα, PIK3CA , serves as an amplifier but not a primary activator of this pathway. This interaction is particularly important in cancerous cells as it serves to amplify basal PI3K activity and support tumor progression. Conversely, in healthy cells, RAS dependent amplification of PI3K signaling is expendable as RAS independent activation of PI3K by upstream signaling factors is sufficient for maintaining physiological homeostasis . Unfortunately, traditional strategies of targeting the PI3K pathway have been unsuccessful as they do not discriminate between RAS dependent and RAS independent signaling. This leads to on-target dose-limiting toxicities, most commonly hyperglycemia and rash.

Vividion has discovered small molecules that disrupt the RAS:PIK3CA interaction through covalent ligation of C242, in the RAS binding domain, adjacent to the RAS binding interface. Using a Nanobit system to measure the RAS:PIK3CA interaction and signaling assays in “RAS active” cells, we have optimized molecules that disrupt the RAS:PIK3CA interaction and inhibit RAS mediated activation of PIK3CA. Further, this unique mechanism of action does not impact glucose handling in mice, contrary to the effects seen when mice are dosed with PIK3CA active site inhibitors. Our RAS:PIK3CA inhibitors are effective and well tolerated in a variety of RAS dependent models, with profound efficacy when used in combination with an agent targeting the MAPK pathway or with a therapy directly targeting mutant KRAS. Finally, we found that ligation of C242 on PIK3CA blocks HER2/3 driven activation of PI3Kα in a RAS independent manner. Overall, our data supports the clinical investigation of these molecules, particularly in combination with rationally chosen therapies where they may provide a tolerable and efficacious means of blocking the PI3K pathway .

Introduction



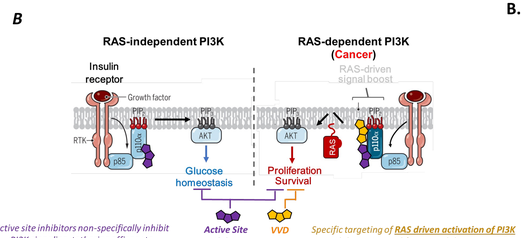


Figure 1:

A. Genetic disruption of the RAS-PI3Kα interaction inhibits progression of KRAS-G12D driven lung cancer in mice[1]. Importantly, although the RAS-PI3Kα interaction is also disrupted in the healthy tissue of these animals, there was no impact on the fitness of these animals. This suggests that hyperactivated RAS amplifies PI3Kα signaling in transformed cells, while the interaction is dispensable in healthy tissue.

B. Current therapies targeting PI3Kα are active site inhibitors, that block all kinase activity of the enzyme. This lack of tumor selectivity results in a small therapeutic window and has prevented their use in RAS activated cancers. Alpelisib, the only clinically approved PI3Kα inhibitor, successfully treats PI3Kα mutant breast patients. In this case, the therapeutic window is generated through extreme dependence of the cancer cells on the PI3K/AKT pathway relative to healthy tissue. Nonetheless, achieving meaningful efficacy prior to hitting DLTs remains as a challenge as dose reductions/holidays are commonly seen in patients receiving alpelisib.

[1]Castellano et al. Requirement for interaction of PI3-Kinase p110α with RAS in Lung Tumor Maintenance. Cancer Cell. 2013.

[2]Gupta et al. Binding of RAS to Phosphoinositide 3-Kinase p110α is Required for Ras-Driven Tumorigenesis. Cell. 2013.

Results

Ligation of C242, adjacent to the RAS binding domain(RBD) on p110α, disrupts the interaction between RAS and p110α.

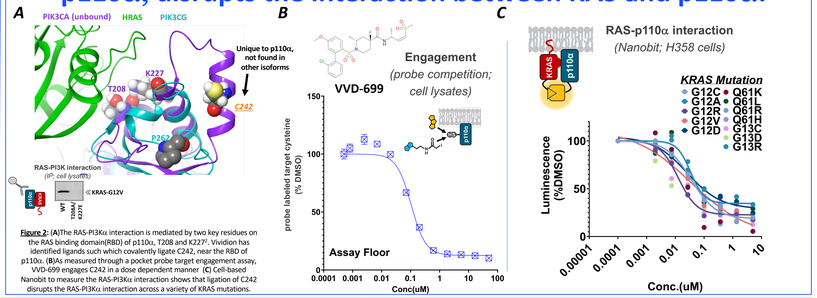


Figure 2: (A) The RAS-PI3Kα interaction is mediated by two key residues on the RAS binding domain(RBD) of p110α, T208 and K227[2]. Vividion has identified ligands such which covalently ligate C242, near the RBD of p110α. **(B)**As measured through a pocket probe target engagement assay, VVD-699 engages C242 in a dose dependent manner **(C)** Cell-based Nanobit to measure the RAS-PI3Kα interaction shows that ligation of C242 disrupts the RAS-PI3Kα interaction across a variety of KRAS mutations.

Disruption of the RAS-p110α interaction blocks PI3K signaling in RAS active cells

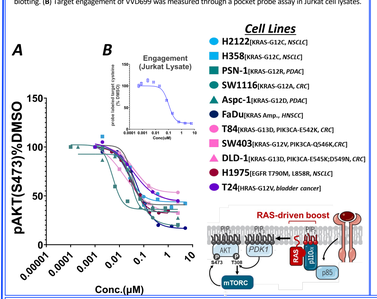


Figure 3: (A)"RAS Driven" cells were treated with a dose response of VVD-699 for 2 or 4 hours. Cells were then harvested and phosphorylation of AKT on residue S473 was measured through HTRF or ProteinSimple western blotting. **(B)** Target engagement of VVD699 was measured through a pocket probe assay in Jurkat cell lysates.

Ligation of p110α blocks HER2/3 dependent activation of PI3Kα independent of RAS

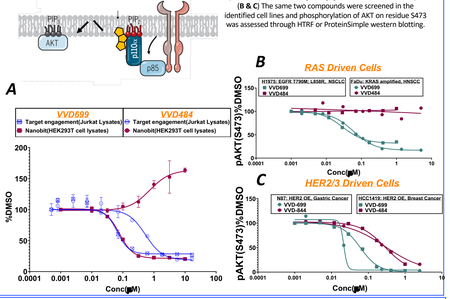


Figure 4: (A) VVD-699 and VVD-484 were included in the previously described target engagement and Nanobit assays. **(B & C)** The same two compounds were screened in the identified cell lines and phosphorylation of AKT on residue S473 was assessed through HTRF or ProteinSimple western blotting.

VVD ligands do not impact intrinsic kinase activity and do not affect RAS-independent PI3K signaling required for glucose homeostasis

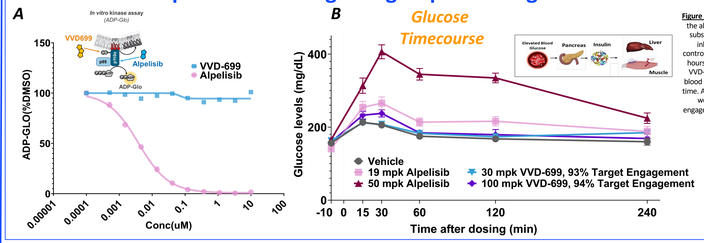
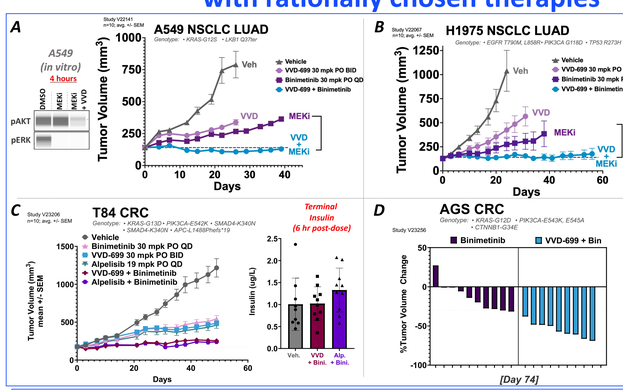


Figure 5: (A) *In vitro* kinase assay assessing the ability of PI3Kα to phosphorylate its substrate PIP_2. Alpelisib, an active site inhibitor, is included as a positive control**(B)** C57BL/6 mice were fasted for 3 hours and then orally dosed alpelisib or VVD-699 at the dose levels shown and blood glucose levels were measured over time. After the final glucose reading, livers were collected and p110α target engagement with VVD-699 was evaluated through mass spectrometry.

VVD RAS-PI3K inhibitors show strong efficacy in combination with rationally chosen therapies



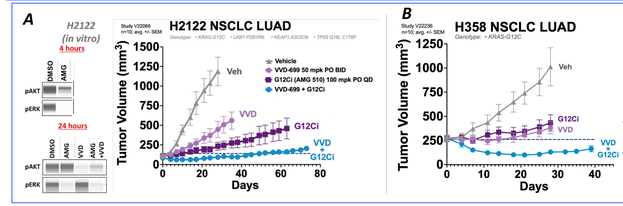


Figure 6: (A-D) *In vivo* efficacy of VVD-699, Binimetinib and a combination of the therapies was tested in the identified models. **(A)**A549 cells were treated *in vitro* with Trametinib(MEKi) alone or in combination with VVD699. Activation of the PI3K & MAPK pathways were assessed through pAKT & pERK, respectively. (C) Plasma insulin levels 6 hours post last dose were measured through ELISA for the indicated groups.

VVD + MEKi

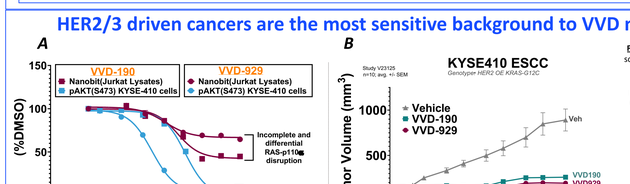


Figure 7: (A & B) *In vivo* efficacy of VVD-699, AMG-510(G12Ci) and a combination of the therapies was tested in the identified models.
(A)H2122 cells were treated *in vitro* with AMG-510(G12Ci) alone or in combination with VVD-699. Activation of the PI3K & MAPK pathways were assessed through pAKT & pERK, respectively.

VVD + G12Ci

HER2/3 driven cancers are the most sensitive background to VVD monotherapy

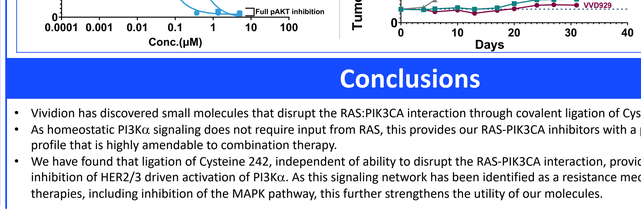


Figure 8: (A) The identified compounds were screened in our RAS-p110α Nanobit assay and cell-based compound performance was assessed in KYSE-410 cells, analyzing phosphorylation of AKT on S473. (B) The *in vivo* efficacy of the same compounds were then assessed in the KYSE-410 model.

Conclusions

- Vividion has discovered small molecules that disrupt the RAS:PIK3CA interaction through covalent ligation of Cysteine 242
- As homeostatic PI3Kα signaling does not require input from RAS, this provides our RAS-PIK3CA inhibitors with a potentially unique safety profile that is highly amendable to combination therapy.
- We have found that ligation of Cysteine 242, independent of ability to disrupt the RAS-PIK3CA interaction, provides near complete inhibition of HER2/3 driven activation of PI3Kα. As this signaling network has been identified as a resistance mechanism to many therapies, including inhibition of the MAPK pathway, this further strengthens the utility of our molecules.