IK-595, A MEK/RAF COMPLEX INHIBITOR, OBVIATES CRAF MEDIATED RESISTANCE RESULTING IN SUPERIOR RAS/MAPK PATHWAY INHIBITION AND ANTI-TUMOR ACTIVITY IN RAS/RAF ALTERED CANCERS

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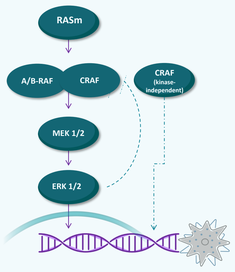
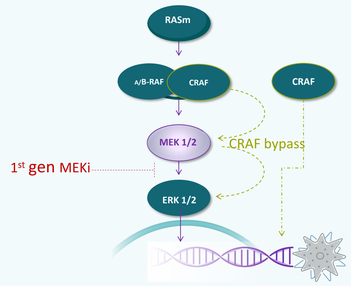
Background

MEK's role in driving ERK-mediated tumor growth[1-4]

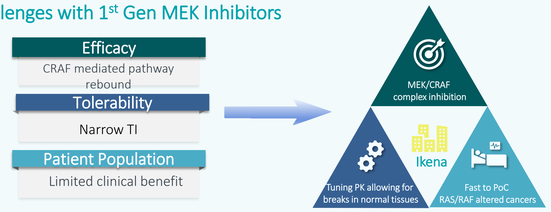


First gen MEK inhibitors trigger CRAF mediated pathway reactivation



- Approved MEK inhibitors block MEK kinase activity
- ERK dependent negative feedback triggers CRAF mediated pathway reactivation
- CRAF was reported to have kinase-independent anti-apoptotic activities that will be completely missed by 1st gen MEK inhibitors
- CRAF plays a critical role in the therapeutic resistance of approved MEK inhibitors in RAS mutant cancer patients

Challenges with 1st Gen MEK Inhibitors



Results

IK-595 Stabilizes MEK-RAF Complex in an Inactive Conformation

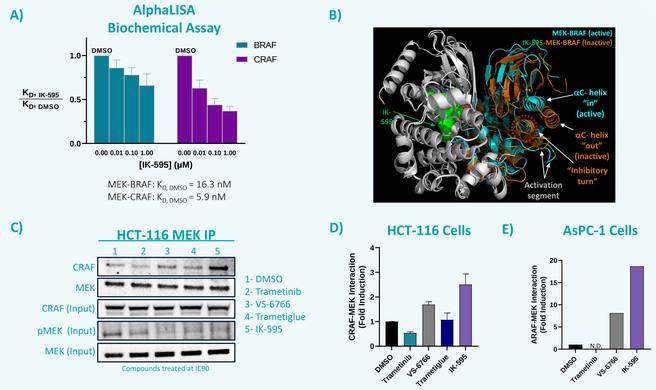


Figure 1. A) Biochemical AlphaLISA assay demonstrating a dose-dependent stabilization of the MEK-CRAF and MEK-BRAF complexes in response to IK-595 treatment. B) Crystal structure of IK-595 in complex with BRAF and MEK. IK-595 induces αC-helix "out" inactive conformation in BRAF protein. C) Enhanced co-immunoprecipitation of MEK and CRAF in response to IK-595 at 4 hours in HCT-116 cells compared to trametinib , VS-6766, and Trametiglue. D) Quantification of MEK-CRAF co-immunoprecipitations in HCT-116 from (C). E) Mass spectrometry of MEK immunoprecipitates demonstrates MEK-ARAF stabilization by IK-595 compared to trametinib and VS-6766.

IK-595 Inhibits MEK Phosphorylation

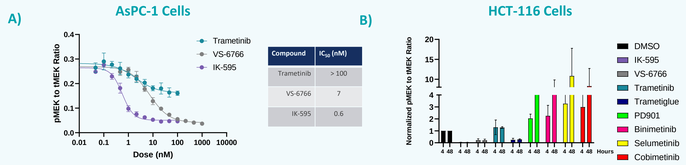


Figure 2. A) MEK phosphorylation by HTRF assay demonstrates potent inhibition of MEK phosphorylation at 4 hours by IK-595 compared to trametinib and VS-6766. B) Quantification of Phospho-MEK1/2 Western blots in HCT-116 demonstrating that IK-595 blocks MEK phosphorylation for up to 48 hours, whereas 1st Gen MEK inhibitors lead to enhanced MEK phosphorylation.

IK-595 Demonstrates Prolonged and Robust Inhibition of ERK1/2 Phosphorylation

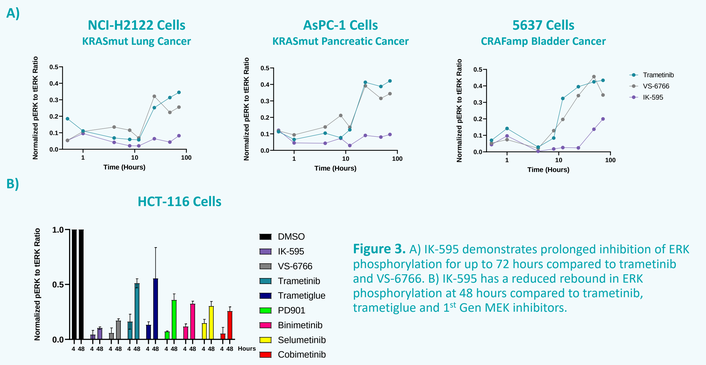


Figure 3. A) IK-595 demonstrates prolonged inhibition of ERK phosphorylation for up to 72 hours compared to trametinib and VS-6766. B) IK-595 has a reduced rebound in ERK phosphorylation at 48 hours compared to trametinib, trametiglue and 1st Gen MEK inhibitors.

Increased Sensitivity to IK-595 in KRAS- and NRAS-mutant and CRAF-Altered Cell Lines

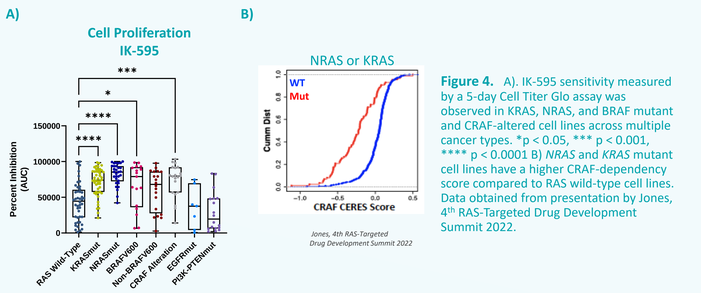


Figure 4. A). IK-595 sensitivity measured by a 5-day Cell Titer Glo assay was observed in KRAS, NRAS, and BRAF mutant and CRAF-altered cell lines across multiple cancer types. *p < 0.05, *** p < 0.001, **** p < 0.0001 B) *NRAS* and *KRAS* mutant cell lines have a higher CRAF-dependency score compared to RAS wild-type cell lines. Data obtained from presentation by Jones, 4th RAS-Targeted Drug Development Summit 2022.

IK-595 Synergizes with Multiple Combination Agents

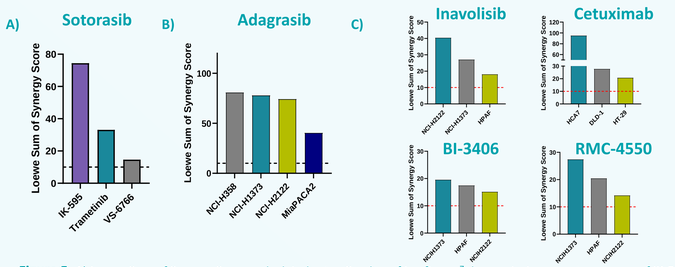


Figure 5. A) Loewe Sum of Synergy Scores calculated using Combenefit software[7] demonstrates superior synergy of IK-595 with sotorasib compared to trametinib and VS-6766. B) IK-595 synergizes with adagrasib across a cell line panel. C) IK-595 synergizes with PI3K (Top Left), EGFR (Top Right), SOS1 (Bottom Left), and SHP2 (Bottom Right) inhibitors.

IK-595 Demonstrates Robust Anti-Tumor Activity Across RAS/RAF-Altered In Vivo Models

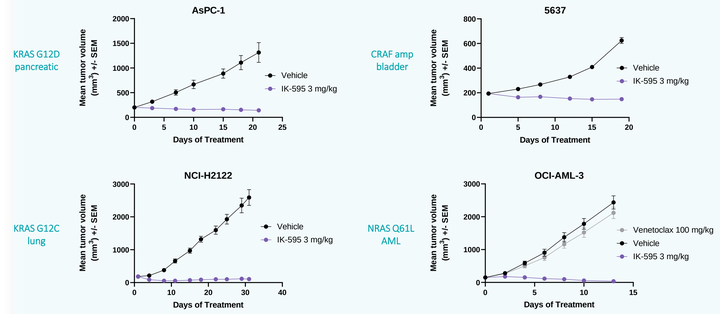


Figure 6. IK-595 demonstrates tumor regressions in all RAS/RAF-altered in vivo tumor models.

Projected Human $T_{1/2}$ of IK-595 Enables Transient IC_{90} Coverage to Maximize Tolerability

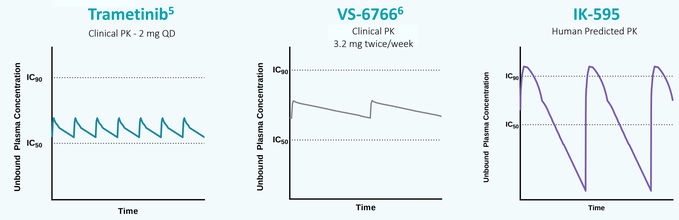
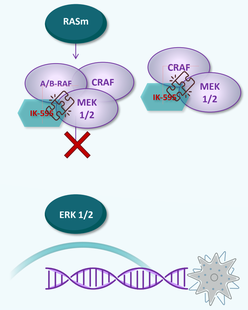


Figure 7. Left panel – Clinical pharmacokinetics of trametinib at steady state ($T_{1/2}$ = 72-120 hours). Middle panel - Clinical pharmacokinetics of VS-6766 at steady state ($T_{1/2}$ = 60-100 hours). Right panel – Predicted human pharmacokinetics of IK-595 with shorter $T_{1/2}$ allowing for transient IC_{90} coverage followed by periods of normal tissue recovery.

Conclusions

IK-595 traps MEK & RAF in an inactive complex to prevent CRAF bypass and kinase-independent CRAF function



IK-595 is designed to and has shown preclinical evidence of superior profile compared with first generation and in-development MEK inhibitors

- ✓Inhibits MEK mediated ERK1/2 phosphorylation
- ✓Prevents MEK phosphorylation by RAF
- ✓Alleviates therapeutic resistance through CRAF mediated bypass and pathway reactivation
- ✓Optimized PK profile to target IC_{90} plasma concentrations widening the therapeutic window
- ✓Combines synergistically with therapies targeting RAS or other compensatory pathways
- ✓IND submission targeted in 2H 2023
- ✓FiH study planned to target selected subsets of CRAF dependent, NRASm and KRASm tumors with significant unmet medical need for monotherapy and combination opportunities

References

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4. Nolan *et al.* Genes 2021.
5. Infante *et al.* The Lancet Oncology 2012.
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