IK-595, a best-in-class MEK-RAF complex inhibitor, drives broad and potent anti-tumor activity in RAS/RAF-driven tumors

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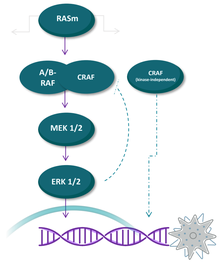
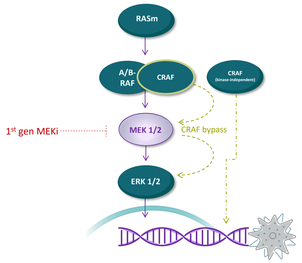
Background

MEK's role in driving ERK-mediated tumor growth



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 missed by 1st gen MEK inhibitors
- **CRAF plays a critical role in the therapeutic resistance of approved MEK inhibitors in RAS mutant cancer patients**

Large Unmet Medical Need Drives Development of Next-Gen MEK-RAF Inhibitors

With broad activity in cancers with KRAS, NRAS, RAF, and NF1 mutations and RAF fusions

With optimized PK profile allowing for improved TI and intra- and inter-pathway combination opportunities

An Effective Next-Gen MEK-RAF Inhibitor Could be Broadly Impactful in RAS/RAF-Altered Patient Population

Results

IK-595, a Potential Best-In-Class Next-Gen Inhibitor, Stabilizes MEK-RAF Complex in an Inactive Conformation

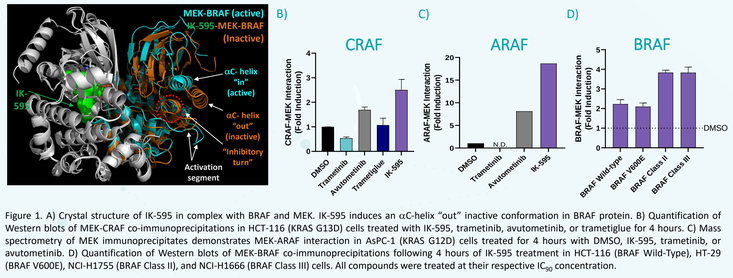


Figure 1. A) Crystal structure of IK-595 in complex with BRAF and MEK. IK-595 induces an αC-helix "out" inactive conformation in BRAF protein. B) Quantification of Western blots of MEK-CRAF co-immunoprecipitations in HCT-116 (KRAS G13D) cells treated with IK-595, trametinib, avutometinib, or trametiglue for 4 hours. C) Mass spectrometry of MEK immunoprecipitates demonstrates MEK-ARAF interaction in AsPC-1 (KRAS G12D) cells treated for 4 hours with DMSO, IK-595, trametinib, or avutometinib. D) Quantification of Western blots of MEK-BRAF co-immunoprecipitations following 4 hours of IK-595 treatment in HCT-116 (BRAF Wild-Type), HT-29 (BRAF V600E), NCI-H1755 (BRAF Class II), and NCI-H1666 (BRAF Class III) cells. All compounds were treated at their respective IC_{50} concentration.

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

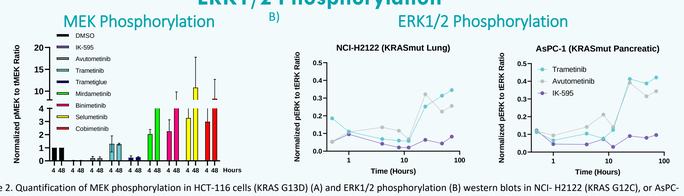


Figure 2. Quantification of MEK phosphorylation in HCT-116 cells (KRAS G13D) (A) and ERK1/2 phosphorylation (B) western blots in NCI- H2122 (KRAS G12C), or AsPC-1 (KRAS G12D) cells demonstrates that IK-595 blocks MEK phosphorylation for at least 48 hours and has reduced rebound in ERK phosphorylation for at least 96 hours compared to other MEK inhibitors. All compounds were treated at their respective IC_{90} concentration.

IK-595 Binds to MEK with a Very Slow Off-Rate

SPR Single Cycle Kinetics

MEK	$k_{on}(M^{-1}s^{-1})$	$k_{off}(s^{-1})$	K_D (nM)
IK-595 (to MEK)	8.24 E+04	6.09 E-04	7.39

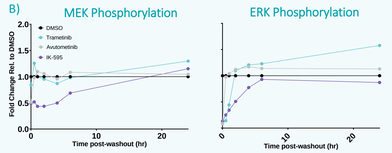


Figure 3. A) IK-595 has a slow off-rate to unphosphorylated MEK1 as measured by SPR. B) IK-595 demonstrates prolonged inhibition of MEK and ERK phosphorylation in HCT-116 cells following compound washout compared to trametinib and avutometinib.

Increased Sensitivity to IK-595 in RAS/RAF-Altered Cell Lines

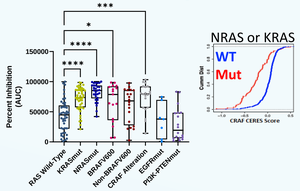


Figure 4. Left Panel - IK-595 sensitivity measured by a 5-day Cell Titer Glo assay was observed in KRAS-, NRAS- and BRAF-mutant and CRAF-altered cell lines across multiple cancer indications. * p < 0.05, *** p < 0.001, **** p < 0.0001. Right Panel - NRAS and KRAS/NRAS mutant cell lines have higher CRAF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

IK-595 Demonstrates Robust Anti-Tumor Activity Across RAS/MAPK-Altered *In Vivo* Models Demonstrating Potential Breadth of Applicability

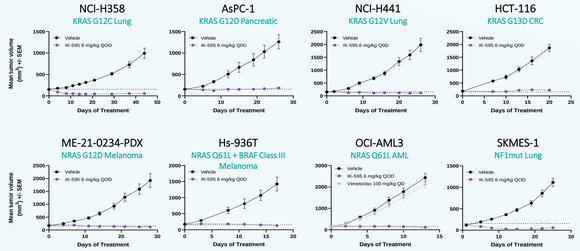


Figure 5. IK-595 demonstrates tumor regressions in all RAS/MAPK pathway-altered *in vivo* tumor models including tumors with KRAS, NRAS, BRAF, and NF1 mutations.

Intermittent Dosing of IK-595 is Efficacious with Superior Tolerability

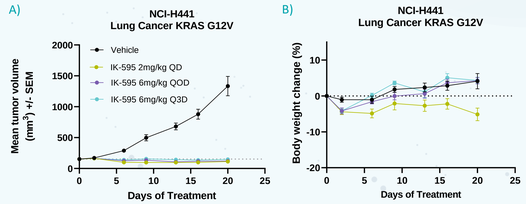


Figure 6. A) IK-595 demonstrates similar efficacy in NCI-H441 CDX model when dosed on a QD, QOD or Q3D regimen. B) Improved tolerability as measured by percent body weight change observed in groups treated with either QOD or Q3D regimen compared to QD dosing.

IK-595 Combinations Broaden Clinical Opportunities

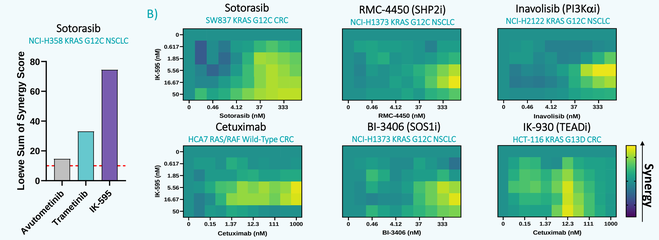
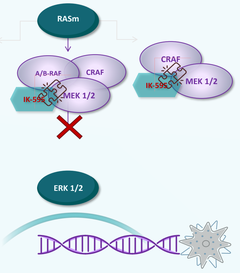


Figure 7. A) IK-595 synergizes with sotorasib stronger than avutometinib and trametinib. B) IK-595 synergizes with sotorasib (KRAS G12C inhibitor), adagrasib (KRAS G12C inhibitor), cetuximab (EGFR monoclonal antibody), RMC-4450 (SHP2 inhibitor), BI-3406 (SOS1 inhibitor), inavolisib (PI3Kalpha inhibitor), and IK-930 (Tead inhibitor). All Loewe Sum of Synergy scores were calculated from a 5-day cell titer glo assay using the Combenefit software.

Conclusion

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



IK-595 has potential to be a best-in-class inhibitor that could broadly impact the RAS therapeutic space

- ✓ Inhibits MEK mediated ERK1/2 phosphorylation
- ✓ Prevents MEK phosphorylation by RAF
- ✓ Alleviates therapeutic resistance through CRAF mediated bypass and pathway reactivation
- ✓ Robust and broad efficacy in RAS/MAPK pathway-altered tumors
- ✓ PK profile enables targeting IC_{90} plasma concentrations and allowing recovery effectively widening the therapeutic window
- ✓ Brain penetrant with demonstrated brain tumor PD
- ✓ Combines synergistically with therapies targeting RAS or other compensatory pathways