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Exarafenib (KIN-2787) is a potent, selective pan-RAF inhibitor with activity in preclinical models of BRAF Class II/III mutant and NRAS mutant melanoma

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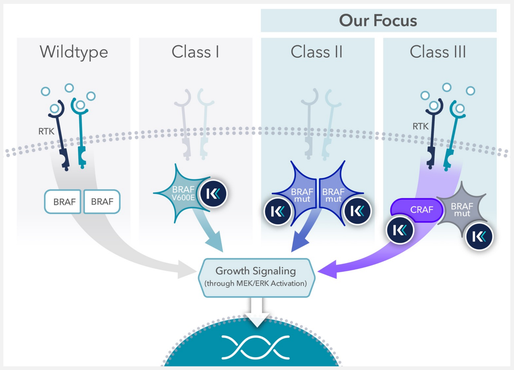
BACKGROUND

Three classes of BRAF alterations:

Class I - active kinase signaling of BRAF mutant monomers (BRAF^{V600x})

Class II - active kinase signaling of BRAF mutant homodimers

Class III – kinase-impaired mutant BRAF amplified via RAS-dependent, RAF heterodimers



- MAPK activating mutations are common in melanoma with 40% of cases attributed to oncogenic BRAF mutations and 20-25% to NRAS mutations
- BRAF^{V600E} targeted inhibitors in combination with MEK inhibitors are approved for Class I BRAF melanoma, but no RAF-targeted therapies are approved for Class II/III BRAF or NRAS mutant melanoma
- Resistance to current BRAF therapies commonly occurs through reactivation of MAPK signaling via NRAS mutations (approximately 17%) or BRAF dimer-dependent (approximately 24%) mechanisms (*Johnson et al 2015*)
- Pan-RAF + MEK inhibitor combination may benefit MAPK driven melanoma

Exarafenib (KIN-2787) is a novel, selective, small molecule pan-RAF inhibitor designed to be effective in RAF-dependent cancers, including all classes of BRAF alterations

METHODS

- Biochemical kinase inhibition activity of indicated compounds was assessed in radiometric kinase assay at Reaction Biology Corp. in % inhibition at 1 μM and in dose response to generate IC_{50}'s. On target RAF and off target activity of compounds shown was measured at 1 μM and 10 μM top dose, respectively, and IC_{50}'s calculated using 4-parameter fit model.
- Ba/F3 BRAF mutant panel was assessed at KYinno Biotechnology Co. LTD in a CellTiter Glo (CTG) assay following 3 days treatment in triplicate dose response curves at 10 μM top dose and IC_{50}'s calculated using 4-parameter fit model.
- Cellular MAPK & growth inhibition was evaluated across human tumor cell lines and binned into BRAF or RAS mutant status. Box plots show mean EC_{50}'s for individual cell lines with n≥3 and line at median. pERK HTRF and CTG values are following 1h treatment and 5 days of treatment, respectively. EC_{50}'s calculated using 4-parameter fit model.
- Combination benefit of exarafenib + binimetinib was tested in 9 x 5 well format, 7 day CTG assays in duplicate. Drug synergy calculated with models using MuSyC and Combenefit. MuSyC = Multidimensional Synergy of Combinations. Loewe model of drug synergy mapped onto dose response surfaces with Combenefit. (MuSyC: Meyer, Cell Syst, 2019) (Combenefit: di Veroli, Bioinformatics, 2016).
- *In vivo* efficacy was evaluated in cell line- (CDX) and patient-derived (PDX) xenograft models of BRAF and NRAS mutant melanoma. Exarafenib was orally administered twice daily (BID) at 10, 20, or 30 mg/kg. Binimetinib was orally administered BID at 3 mg/kg. All studies were in BALB/c nude mice except SK-MEL-2 (in NOD SCID). Mean tumor volumes (TV) are shown ± SEM, with insets of mean % change in bodyweight relative to day 0. Waterfall plots: TV_f-TV_0/TV_0 x 100% where TV_f = TV at end of treatment and TV_0 = initial TV.
- *In vivo* PD was assessed at 1, 3, 7, 12, and 24 hours after 3 days of dosing. Biomarker changes were analyzed by pERK/tERK MSD and western blot for pERK, tERK, pRSK, tRSK, EphA2, DUSP6, and GAPDH.

SUMMARY

- Exarafenib is a potent, selective pan-RAF inhibitor with activity against RAF-dependent cancers including human melanoma cells bearing BRAF &/or NRAS mutations
- Exarafenib activity is synergistic when combined with MEK inhibition in human NRAS mutant melanoma cells in dose ranges predicted to be clinically-relevant
- Daily exarafenib + binimetinib treatment demonstrates combination benefit in tumor growth inhibition and durable suppression of MAPK pathway in human cell line-derived and patient-derived xenograft models of melanoma
- A Phase I clinical trial evaluating the safety and efficacy of exarafenib in monotherapy and in combination with binimetinib in BRAF mutant solid tumors and NRAS mutant melanoma is ongoing (NCT04913285).

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RESULTS

Exarafenib demonstrates exceptional selectivity across the kinome

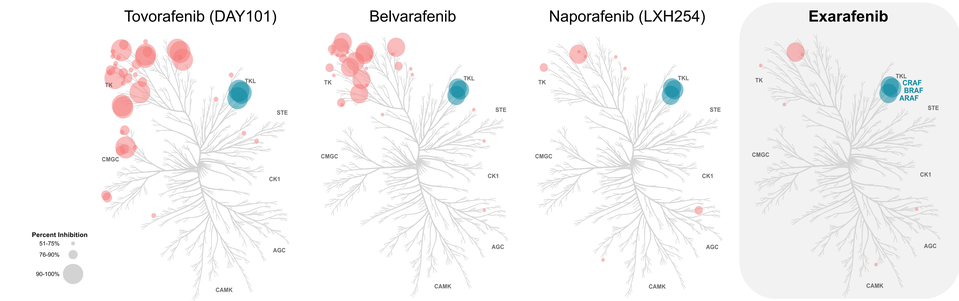

FIGURE 1. Exarafenib kinase % inhibition data at 1 μM relative to investigational pan-RAF inhibitors. Teal circles are ON target RAF kinases. Red circles indicate OFF target kinases with greater size representing greater % inhibition. Only wild type kinases pictured.

Exarafenib is a potent & selective pan-RAF kinase inhibitor

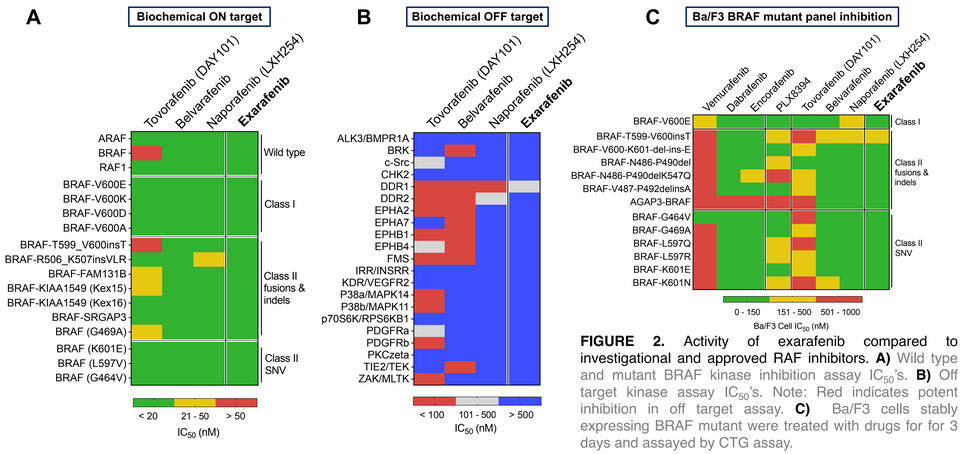

FIGURE 2. Activity of exarafenib compared to investigational and approved RAF inhibitors. **A)** Wild type and mutant BRAF kinase inhibition assay IC_{50}'s. **B)** Off target kinase assay IC_{50}'s. Note: Red indicates potent inhibition in off target assay. **C)** Ba/F3 cells stably expressing BRAF mutant were treated with drugs for for 3 days and assayed by CTG assay.

Exarafenib inhibits MAPK signaling and viability in human melanoma cells

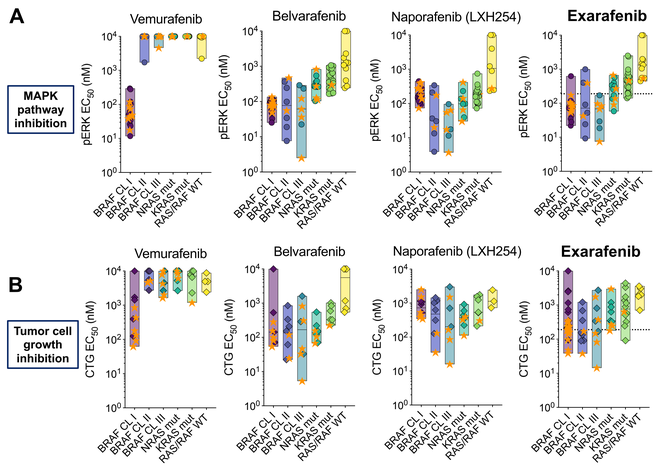

FIGURE 3. Exarafenib activity in BRAF and RAS mutant human tumor cell lines. **A)** Box plots compare MAPK pathway inhibition (pERK EC_{50} values) across human tumor cell lines. Orange stars indicate melanoma cell lines. **B)** Tumor cell growth inhibition was measured by CTG following 5 days of drug treatment. Orange stars indicate melanoma cell lines. **C)** Representative pERK dose response curves in an NRAS mutant melanoma cell line demonstrating lack of paradoxical activation relative to 1st gen BRAF inhibitors.

Exarafenib plus binimetinib combination demonstrates synergy in NRAS mutant melanoma cells

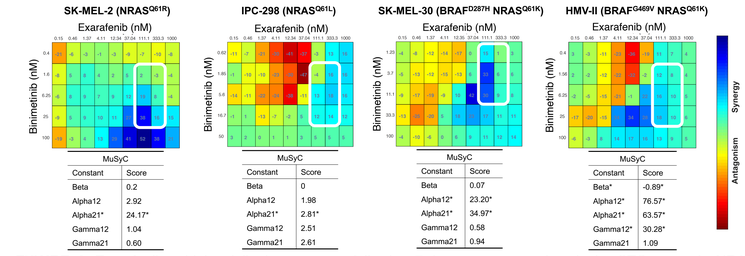

FIGURE 4. Exarafenib + binimetinib drug synergy following 7-day treatment and endpoint CTG assay in NRAS mutant melanoma cell lines. Exarafenib was kept at 1 μM top dose and binimetinib either at 100 or 50 nM top dose. Synergy heat maps display Loewe synergy scores with blue representing greater synergy. MuSyC scores indicate global and directional potency and efficacy synergy, with exarafenib = Drug 1 and binimetinib = Drug 2. *significant by 95% confidence interval. White boxes indicate areas of estimated clinically relevant exposure.

Exarafenib plus binimetinib combination is efficacious in xenograft models of BRAF and NRAS mutant human melanoma

FIGURE 5. Exarafenib, binimetinib, and combination treatment of BRAF and NRAS mutant xenograft models at indicated doses and % TGI. **A)** Human CDX melanoma models, n=9/cohort **B)** PDX isolated from Tx-naïve BRAF^{V600E} melanoma patient. *20 mg/kg BID exarafenib. **C)** NRAS^{Q61x} mutant PDX models with associated waterfall plots indicating % change in tumor volume for each mouse on last day of treatment relative to day 0.

Exarafenib plus binimetinib combination extends MAPK pathway suppression in xenograft model of human melanoma

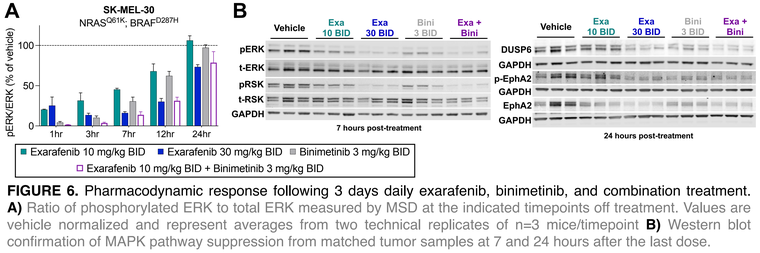

FIGURE 6. Pharmacodynamic response following 3 days daily exarafenib, binimetinib, and combination treatment. **A)** Ratio of phosphorylated ERK to total ERK measured by MSD at the indicated timepoints off treatment. Values are vehicle normalized and represent averages from two technical replicates of n=3 mice/timepoint **B)** Western blot confirmation of MAPK pathway suppression from matched tumor samples at 7 and 24 hours after the last dose.