CFT1946, a potent, selective BRAF V600X mutant-specific degrader demonstrates superior activity as a single agent to clinically approved BRAF inhibitors and standard of care combinations in preclinical models of BRAF V600X melanoma, CRC, NSCLC, and brain metastasis

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#1658

Abstract

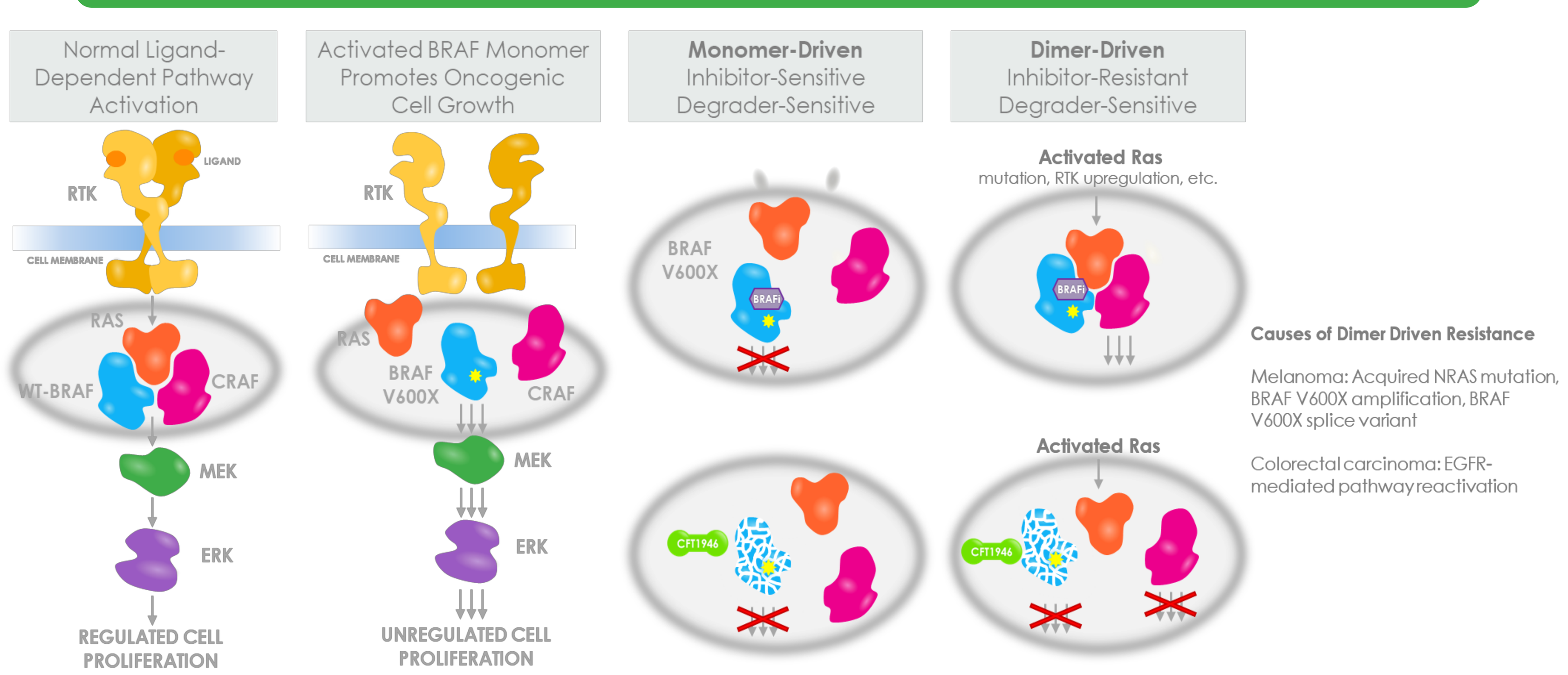
Activating mutations in BRAF at residue V600 (typically V600E) lead to dysregulation of the mitogen-activated protein kinases (MAPK) pathway and occur in approximately 8% of all human cancers, including 60% of melanoma, up to 12% of CRC, and 4% of non-small cell lung cancer (NSCLC). Currently approved BRAF inhibitors (BRAFi) are selective for BRAF V600X mutant proteins and are typically used in combination with MEK inhibitors (MEKi) in melanoma and NSCLC, or anti-EGFR antibodies such as cetuximab in CRC. However, their activity is limited by primary or acquired resistance often mediated by RAF dimer-inducing mechanisms. Furthermore, progression of BRAF V600X melanoma after BRAFi/MEKi treatment frequently involves brain metastasis, and currently approved BRAFi have relatively poor brain penetration.

CFT1946 is a potent, orally bioavailable, cereblon-based BiDAC™ degrader that selectively degrades BRAF V600X mutant protein and is currently under investigation in a Phase I clinical trial. CFT1946, by degrading V600X, abrogates resistance and paradoxical activation associated with RAF dimerization as seen with approved inhibitors. Indeed, we have previously demonstrated that CFT1946 is efficacious in an A375 BRAF V600E/NRAS Q61K xenograft model of BRAFi resistant melanoma (AACR; Cancer Research, (2023) 3425 and (2022) 2158)

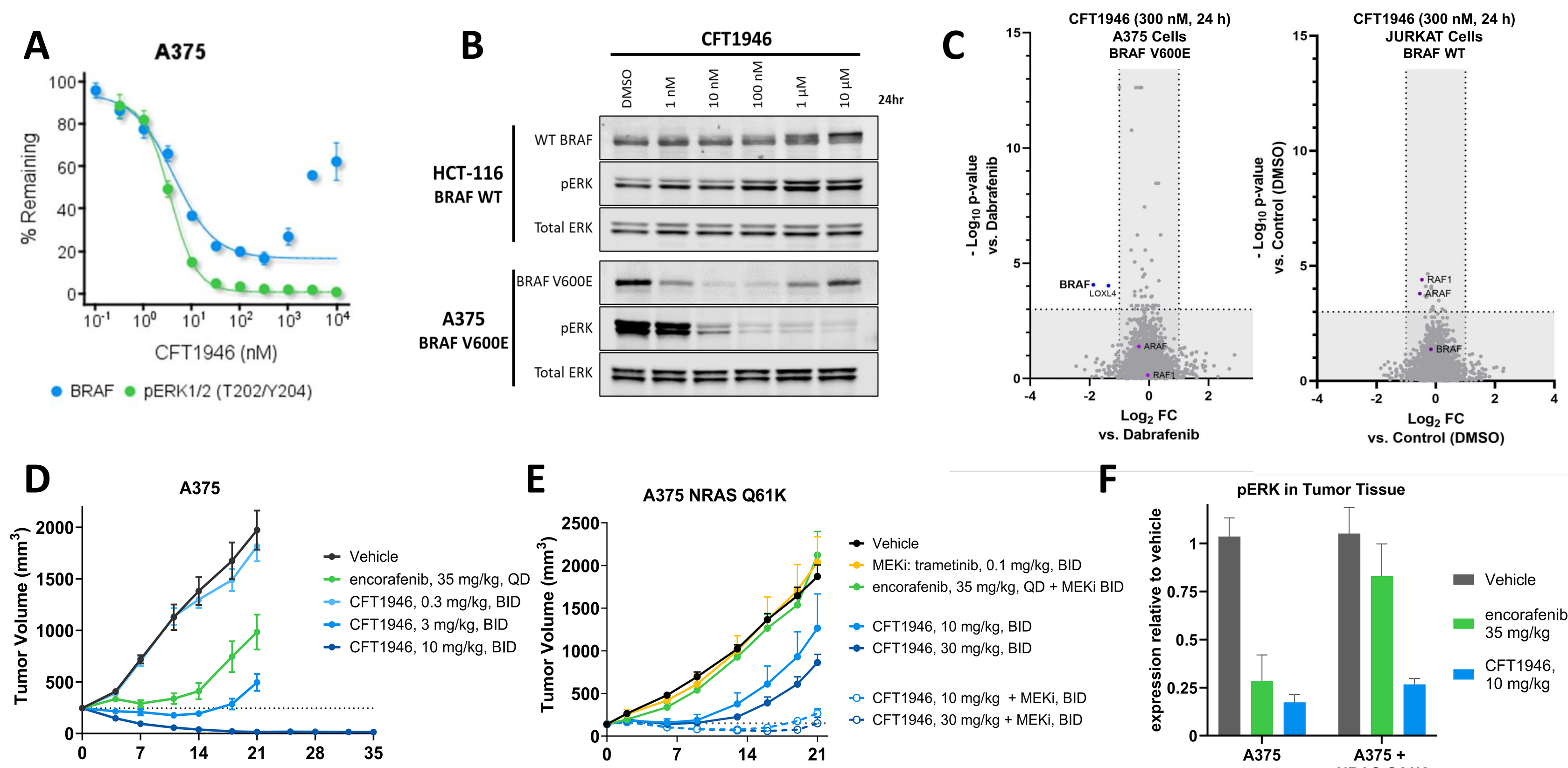
Here we substantially expand our preclinical characterization of CFT1946 across multiple models of BRAF V600X-driven cancers including BRAF V600X-driven CRC and NSCLC, additional BRAFi-resistant melanoma models, and a brain metastatic melanoma model. Single agent CFT1946 outperformed the standard of care (SOC) encorafenib (BRAF inhibitor) + cetuximab (EGFR mAb) combination in a panel of BRAF V600X CRC xenograft models. CFT1946 also demonstrated single agent regression in a BRAF V600X NSCLC PDX model where the SOC dabrafenib (BRAF inhibitor) + trametinib (MEK inhibitor) showed only modest tumor growth inhibition. Consistent with our previous results in the A375 melanoma model, single agent CFT1946 showed superior activity versus the SOC dabrafenib + trametinib combination in all additional melanoma models tested, with complete regression achieved using a CFT1946 + trametinib combination in a PDX model bearing a BRAF V600E kinase duplication that showed minimal response to dabrafenib + trametinib. Finally, using an A375 intracranial model, single agent treatment with CFT1946 gave robust, dose-dependent efficacy and survival advantage over encorafenib.

The promising activity of CFT1946 in a broad range of BRAF V600X preclinical models supports its ongoing clinical investigation in BRAF V600X mutant solid tumors (NCT05668585).

Model for CFT1946 Efficacy in BRAF V600E Monomer and Dimer-Dependent Diseases



CFT1946 is a BRAF Mutant-Specific BiDAC Degrader and is Active in an Inhibitor-Resistant BRAF V600E/NRAS Q61K Melanoma Model



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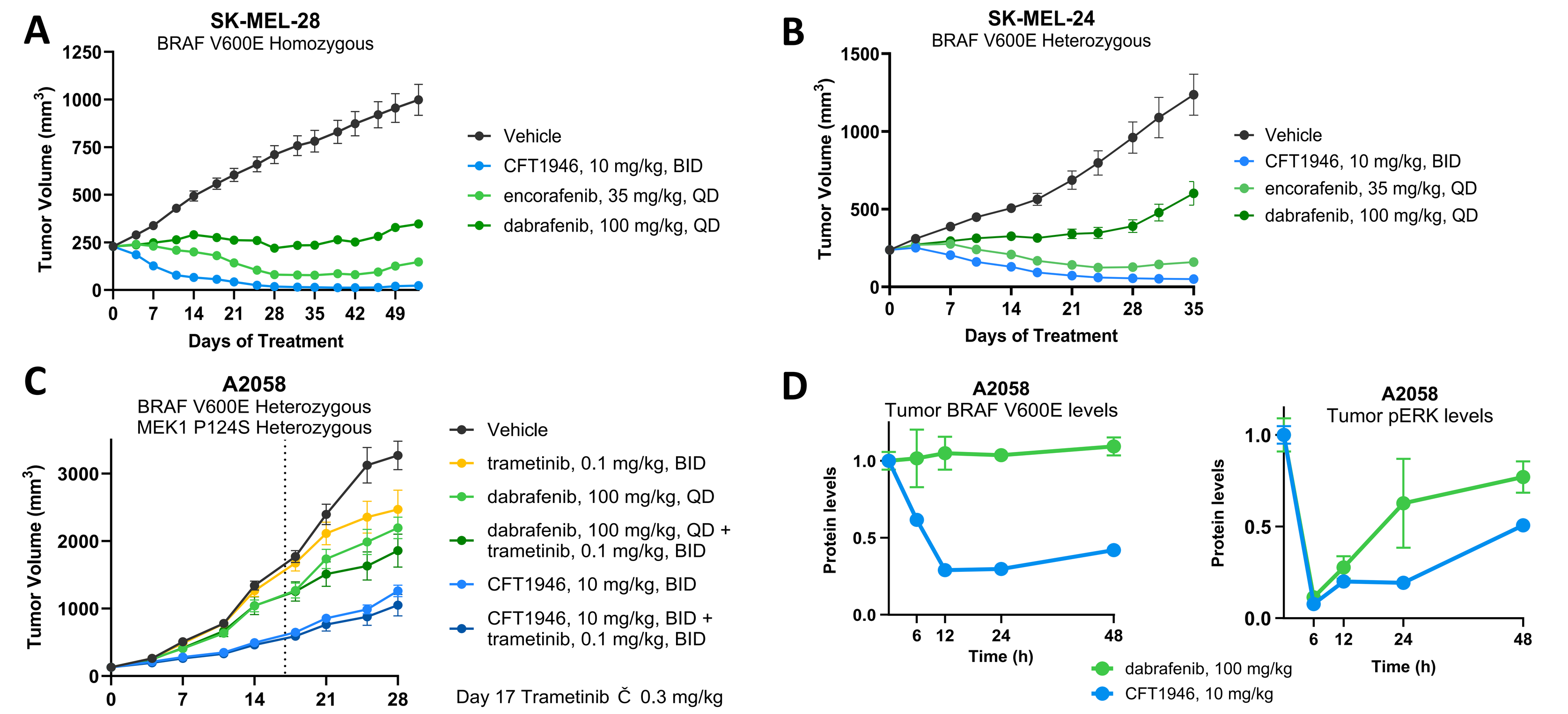
Cell Line	MAPK Status	GI_{50} CFT1946	GI_{50} encorafenib
IMR90	WT	>10000 nM	>10000
HCT-116	KRAS G13D	>10000 nM	>10000
A375	BRAF V600E	6.8 nM	7.5 nM
A375 + NRAS Q61K	BRAF V600E NRAS Q61K	55 nM	911 nM

Figure 1. CFT1946 is a highly specific BRAF mutant-specific degrader that leads to regressions in BRAF V600E melanoma models. (A) CFT1946 concentration-dependent HiBiT-BRAF-V600E degradation with concomitant loss of phospho-ERK (pERK) in A375 cells. Degradation reached an E_{max} of 16% and pERK had an IC_{50} of 3.4 nM. **(B)** Western blot analysis of BRAF WT HCT-116 and BRAF V600E mutant A375 shows selective degradation and pERK suppression only in the mutant setting. **(C)** Proteomic profiling confirms CFT1946-mediated BRAF degradation in BRAF V600E but not WT BRAF cell lines. **(D)** CFT1946 leads to regression in the A375 BRAF V600E homozygous mutant cell derived xenograft (CDX) model. **(E)** CFT1946 + trametinib combination leads to regression in the BRAF inhibitor resistant A375 NRAS Q61K mutant melanoma xenograft model. Compounds in efficacy studies were dosed orally once daily (QD) or twice daily (BID) as indicated. Efficacy data are expressed as mean tumor volumes ± SEM. **(F)** Western blot analysis of pERK at 10 hours post single dose in A375 and A375 NRAS Q61K tumors, normalized to total ERK. **(G)** CellTiter-Glo analysis of cell lines with varying MAPK pathway mutation status shows specificity for mutant setting, and activity in BRAFi resistance setting compared to the BRAF inhibitor encorafenib.

CFT1946 is Superior to Clinically Approved BRAF Inhibitors in Additional BRAF V600E Melanoma CDX and PDX Models



Day 17 Trametinib Č 0.3 mg/kg

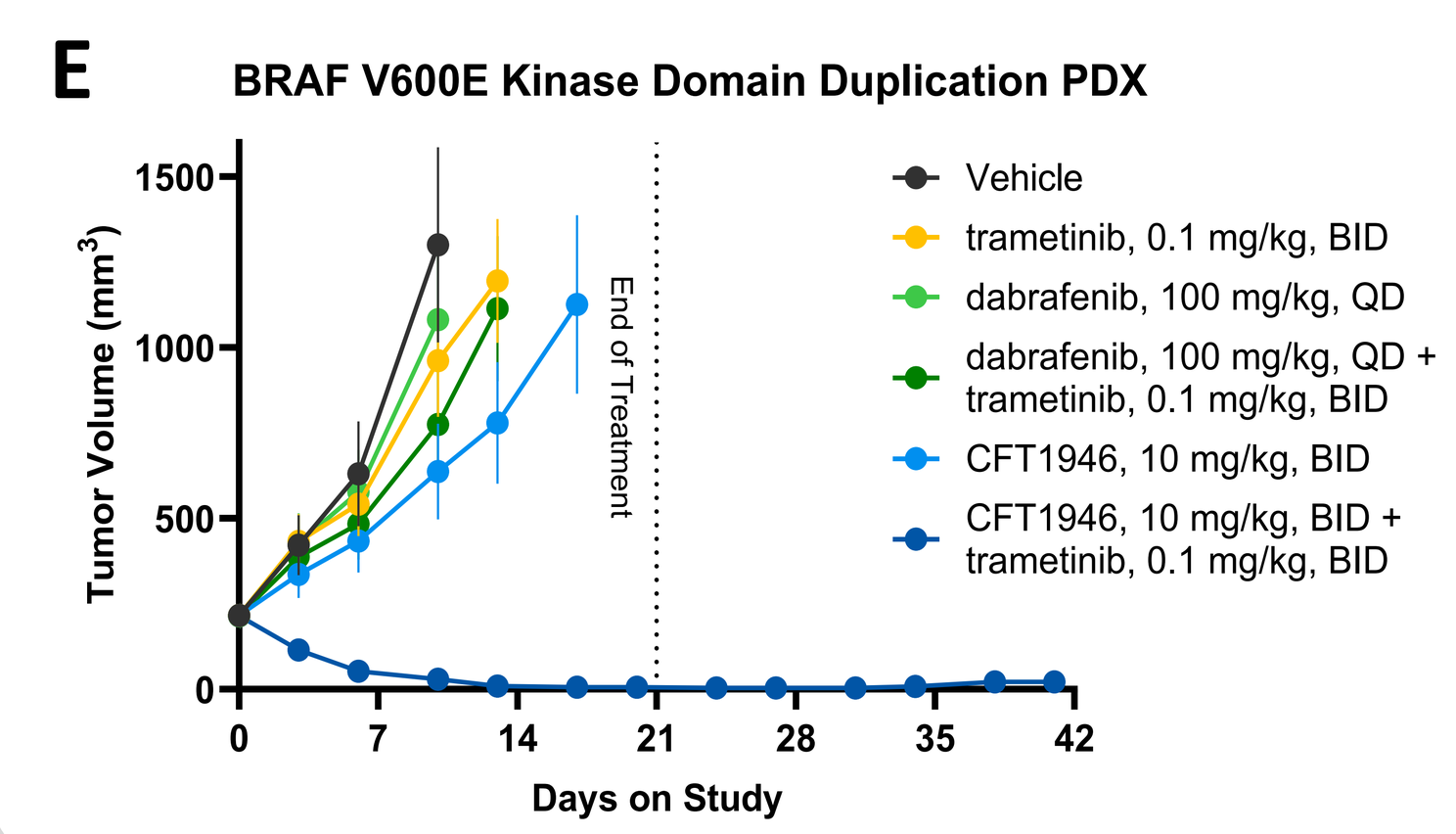


Figure 2. CFT1946 demonstrates efficacy in BRAF V600E mutated melanoma xenograft models. CFT1946 results in greater tumor regression compared to encorafenib and dabrafenib in the **(A)** SK-MEL-28 BRAF V600E homozygous and **(B)** SK-MEL-24 BRAF V600E heterozygous xenograft models. **(C)** Efficacy study of the A2058 BRAF V600E heterozygous melanoma model carrying the activating mutation MEK1 P124S, which retains dependence on upstream RAF scaffolding. At day 17, trametinib was increased to 0.3 mg/kg as indicated by dotted line. CFT1946 inhibited tumor growth to a greater degree as a single agent compared to the dabrafenib and trametinib combination. **(D)** BRAF V600E (left) and phospho-ERK (right) protein levels after a single dose demonstrates target engagement and pathway suppression in the A2058 CDX model. **(E)** PDX ST2870B was established from a patient who progressed on dabrafenib + trametinib combination and acquired a duplication of the V600E mutated BRAF kinase domain. Mice were treated for 21 days as indicated by dotted line. CFT1946 + trametinib combination showed sustained regression after the removal of treatment demonstrating the durability of this response. Compounds in efficacy studies were dosed orally QD or BID as indicated. Efficacy data are expressed as mean tumor volumes ± SEM, n =8 mice per arm.

CFT1946 Leads to Tumor Regression in a NSCLC BRAF V600E PDX Model Where Clinically Approved BRAFi/MEKi Combination is Ineffective

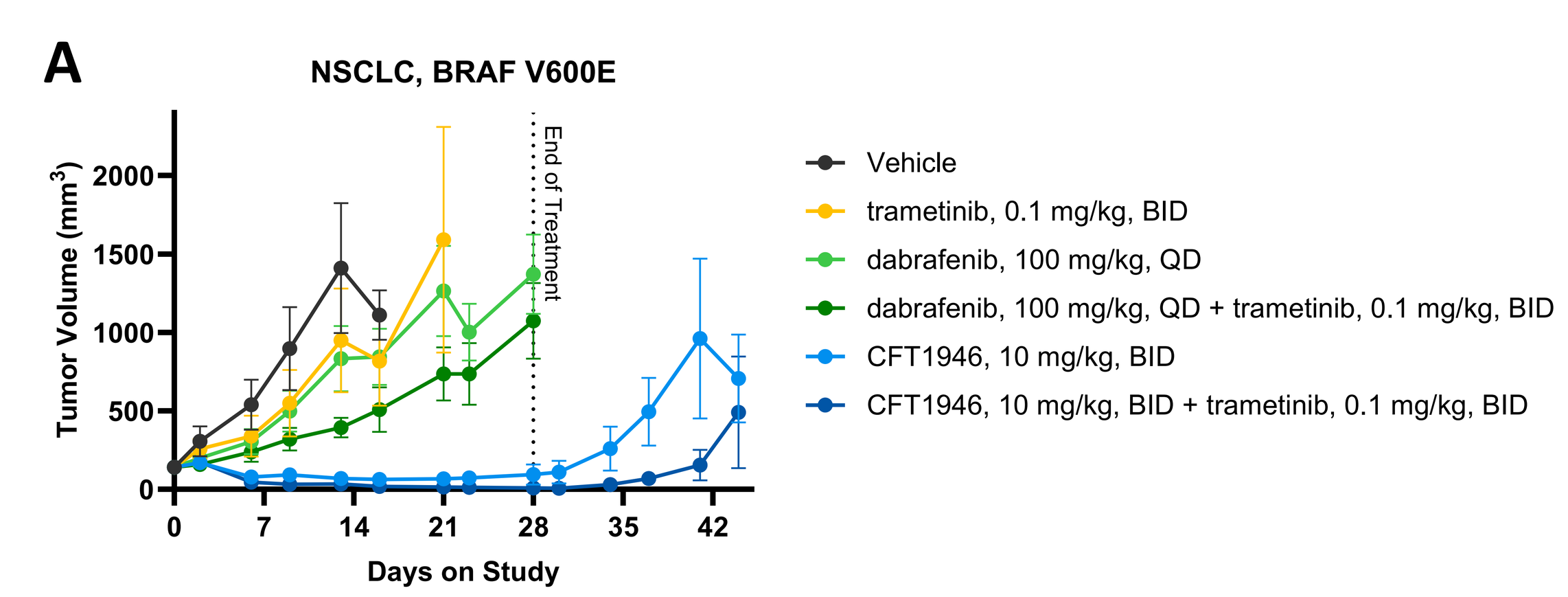
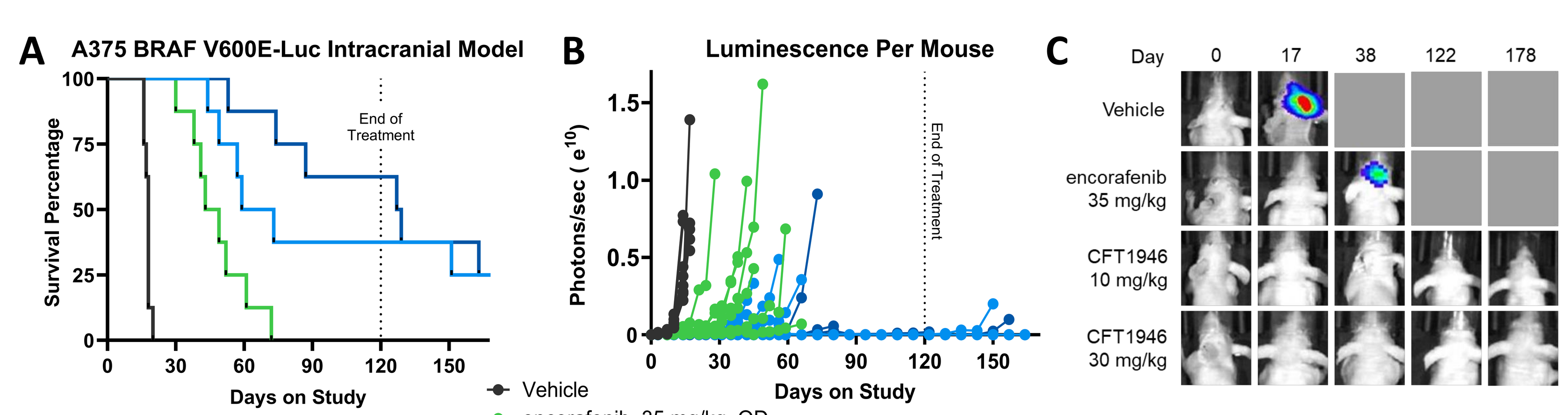


Figure 3. CFT1946 leads to regression as a single agent and in combination with MEK inhibitor in a BRAF V600E mutated NSCLC PDX efficacy study. Both CFT1946 and CFT1946 + trametinib lead to regression with the combination demonstrating a longer durability of response after treatment is stopped on day 28. Tumors grew in the presence of dabrafenib with or without the MEKi combination. Compounds in efficacy studies were dosed orally QD or BID as indicated. Efficacy data are expressed as mean tumor volumes ± SEM, n=8 mice per arm.

CFT1946 Prolongs Survival in an Intracranial Model of BRAF V600E Metastatic Melanoma



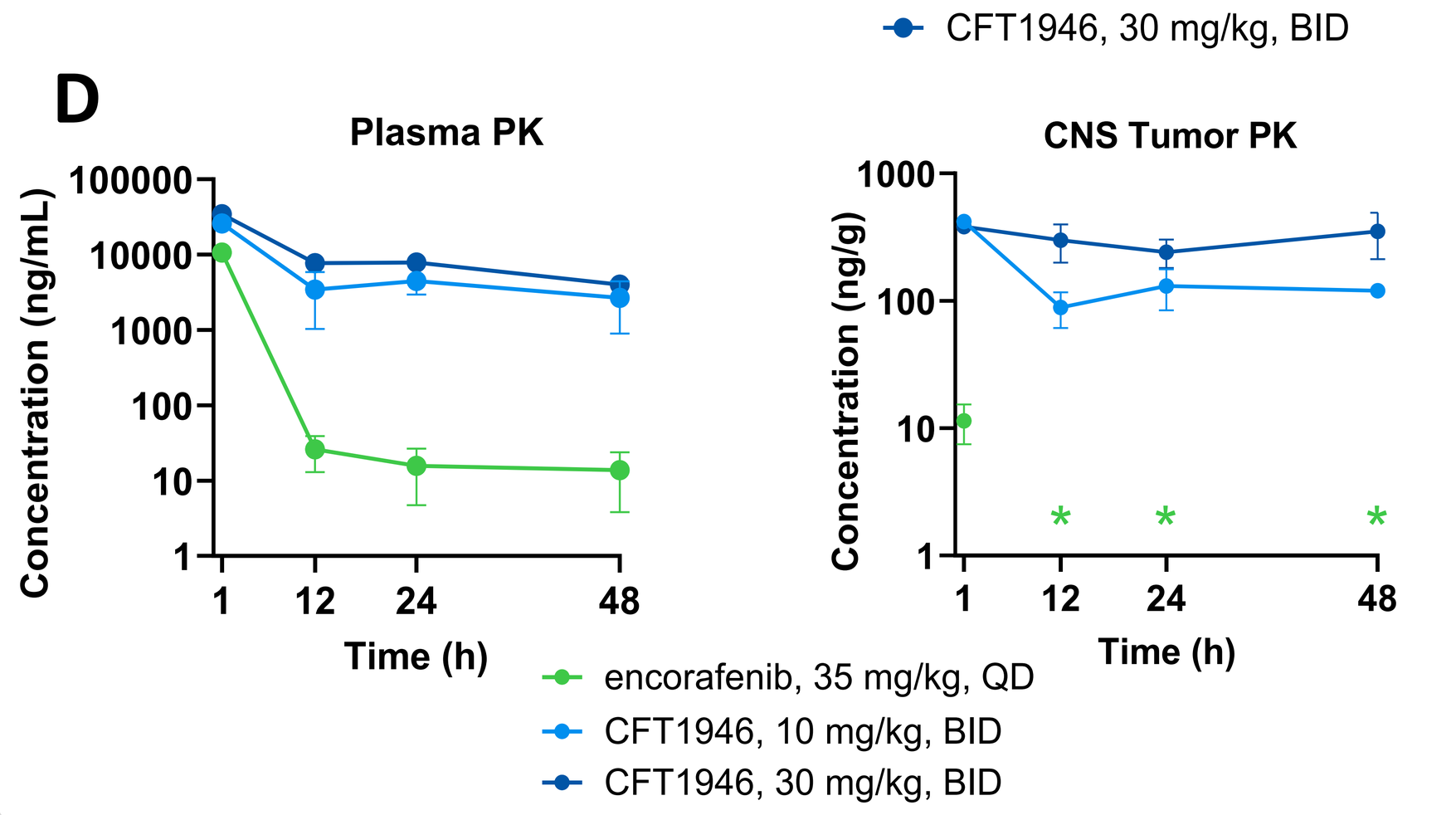
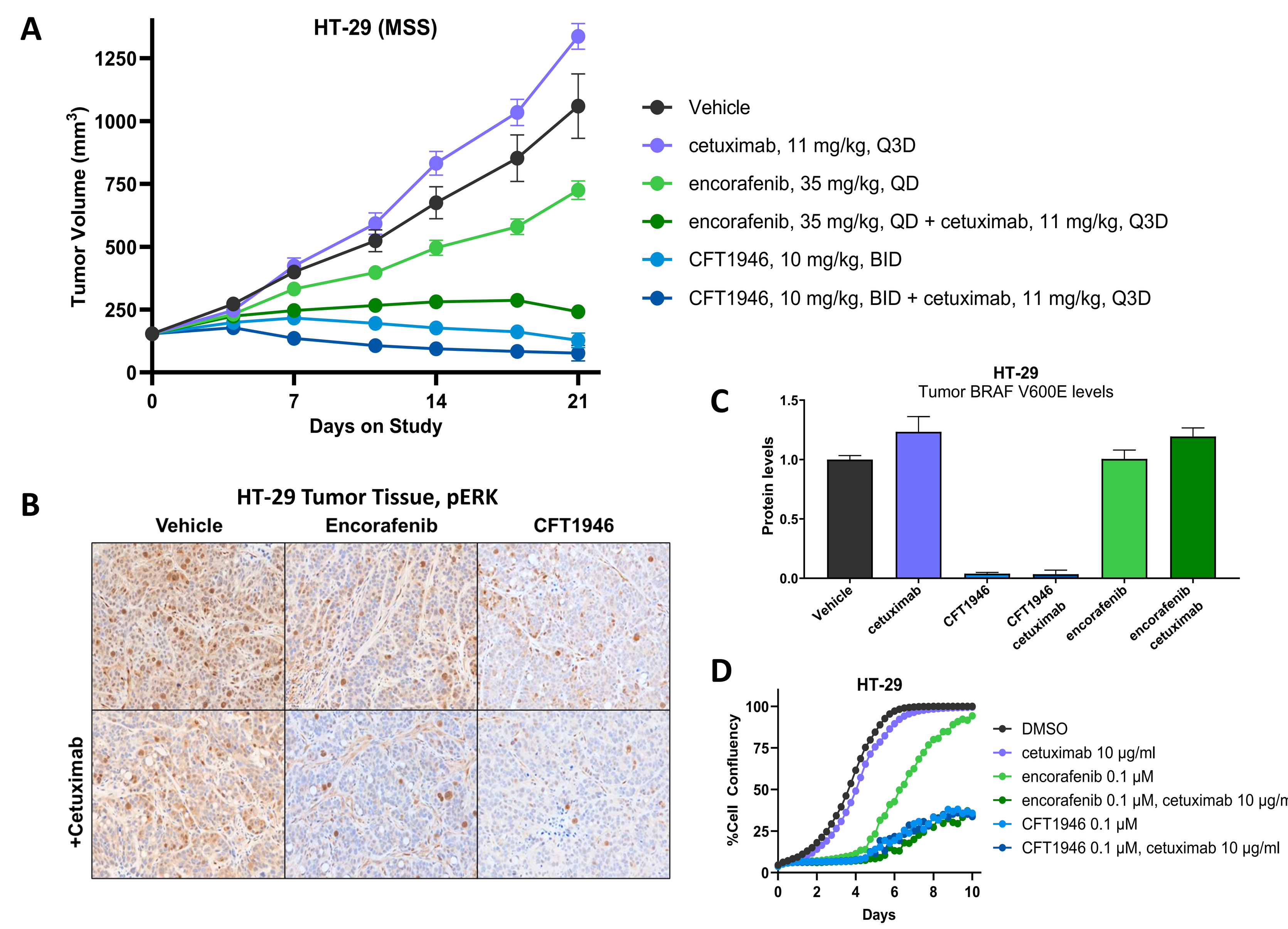


Figure 4. CFT1946 prolongs survival in an intracranial model of metastatic melanoma. (A) Female BALBc/Nude mice underwent intracranial surgical implantation of A375-luciferase cells in 20% Matrigel. Kaplan Meier Survival curve demonstrates survival in response to treatment with encorafenib (35 mg/kg, PO/QD) or CFT1946 (10 mg/kg, or 30 mg/kg, PO/BID). At 120 Days (dotted line) treatment was discontinued, and the remaining mice were monitored for tumor burden, weight loss, and survival. All doses were well-tolerated as no group showed more than mean 10% body weight loss before detectible tumor signal. Mice were terminated when they reached 20% weight loss due to tumor burden. **(B)** Luminescence signal graphed for individual mice on study, representing tumor burden. **(C)** Representative images of luminescence signal, depicting progression of individual mice for each treatment arm at representative timepoints. **(D)** Pharmacokinetics of mouse plasma (left) and brain tumor (right) demonstrate brain exposure of CFT1946 and encorafenib (*encorafenib was below quantifiable level after 1 hour). Compounds in the study were dosed orally QD or BID as indicated, n=8 mice per arm.

BRAF V600E CRC CDX *In Vivo* Models Exhibit Greater Response to CFT1946 in Comparison to Approved BRAFi/EGFRmAb



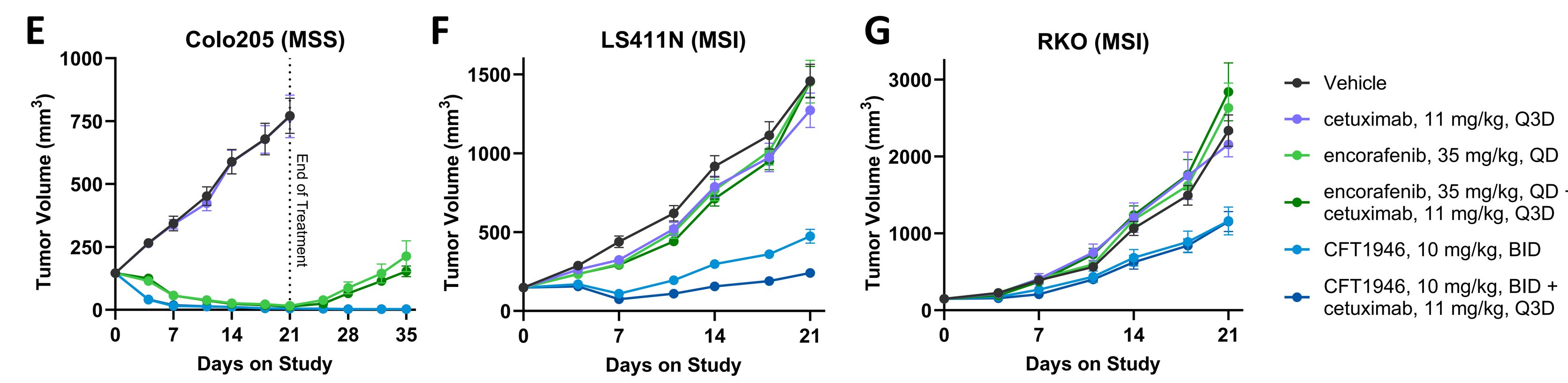


Figure 5. CFT1946 single agent treatment is superior to SOC in CRC models. (A) *In vivo* efficacy of CFT1946 or encorafenib with or without cetuximab combination demonstrates degrader advantage in the BRAF V600E heterozygous colorectal carcinoma model HT-29, microsatellite stable (MSS) model. **(B)** Representative images of tumor tissue collected at end of study at 20x magnification with quantified levels of phosphorylated ERK displayed as H-scores in image panels. CFT1946 treatment results in greater suppression of pERK than the inhibitor or cetuximab alone, CFT1946 in combination with cetuximab has the greatest pERK suppression by IHC, correlating with the deepest level of tumor growth inhibition in the efficacy study. **(C)** Corresponding end of study tumor BRAF V600E protein levels from efficacy study demonstrate BRAF degradation in CFT1946 treated mice. **(D)** Live imaging of cellular confluency over 10 days was captured for HT-29 cells in the presence of CFT1946 or encorafenib with or without cetuximab combination. **(E)** Efficacy study of the BRAF V600E CRC model Colo205, an MSS model known to be sensitive to BRAF inhibitor treatment. Treatment was ceased at day 21 and monitored for outgrowth, CFT1946 treatment resulted in a more durable suppression of tumor growth with tumors still undetectable 14 days after removal of treatment for 7 out of 8 mice. **(F)** *In vivo* efficacy study with the microsatellite instable (MSI) CRC model LS411N demonstrated strong tumor growth inhibition with CFT1946 treatment and further suppression of tumor growth with addition of cetuximab. **(G)** CFT1946 treatment in the MSI CRC model RKO suppressed tumor growth but was not further benefited by addition of cetuximab. Compounds in efficacy studies were dosed orally QD, Q3D, or BID as indicated. Efficacy data are expressed as mean tumor volumes ± SEM.

Summary

CFT1946, a potent and selective orally bioavailable BRAF V600X BiDAC degrader, is:

- **superior to SOC** BRAFi treatment in multiple BRAF V600X mutant **melanoma** PDX and CDX models, including BRAFi resistant models
- **superior to SOC** BRAFi/MEKi treatment in a BRAF V600X mutant **NSCLC** PDX model
- **superior to SOC** BRAFi treatment in a BRAF V600X mutant **intracranial metastatic melanoma** model
- **superior to SOC** BRAFi/EGFRmAb treatment in multiple BRAF V600X mutant **CRC** CDX models

Taken together, our data demonstrate that degradation of BRAF V600X mutants by CFT1946, in monomer and dimer-driven settings, offers the potential to treat patients with a broad range of BRAF V600X mutant-driven cancers including melanoma, NSCLC, and CRC.

These data provide encouraging support of the ongoing clinical investigation of CFT1946 in BRAF V600 mutant solid tumors (NCT05668585).

References

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