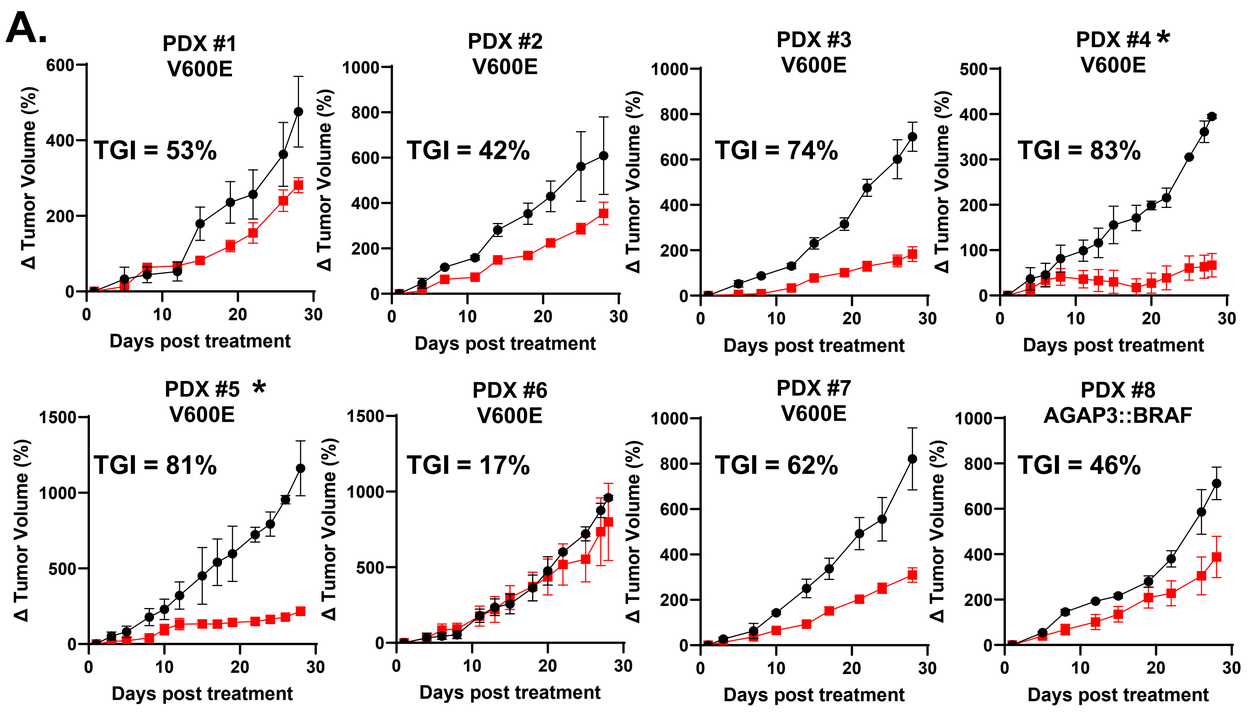
Determinants of response and rational combination strategies for plixorafenib (PLX8394/FORE8394) in models of BRAF-altered colorectal cancer

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Introduction

- BRAF V600 mutation occurs in ~8% of colorectal cancer (CRC), an aggressive tumor with poor prognosis.
- Responses to BRAFi monotherapy in BRAF-altered CRC have been dismal and remain a high unmet need.
- Plixorafenib (FORE8394/PLX8394) is a selective, potent BRAFi that targets mutated BRAF monomers, BRAF homodimers, and BRAF-CRAF heterodimers without inducing RAF dimer formation.
- Plixorafenib inhibits class 1 (V600), class 2, and fusion BRAF alterations.
- Unlike other BRAF inhibitors, plixorafenib evades paradoxical MAPK activation by disrupting BRAF dimer formation and reduces toxicities and intrinsic resistance associated with 1st generation BRAFi (encorafenib, dabrafenib and vemurafenib).
- In a Phase 1/2a study (NCT02012231), anti-tumor activity of plixorafenib was seen across multiple solid tumors, including $BRAF^{V600E}$-altered, MAPKi treatment naïve, CRC (n=13, 7.7% ORR, mPFS of 3.5 months).
- Thus, we wanted to understand mechanisms of response and resistance to plixorafenib treatment in BRAF-altered CRC.

Plixorafenib limits tumor growth in PDX and CDX models of V600E or BRAF fusion altered CRC



* **Prior Treatments. PDX #4 – 5FU+Leucovorin+Enluacil. PDX #5 – FOLFOX, Erbitux, Irinotecan**

Figure 1. Mice were inoculated subcutaneously with 8 PDX (A) or 3 CDX (B) CRC models with V600E or BRAF fusion alterations and treated with 50mg/kg plixorafenib for 21-28 days. Tumor growth was measured throughout treatment.

Genetic alterations that may predict response/resistance to plixorafenib as a single agent

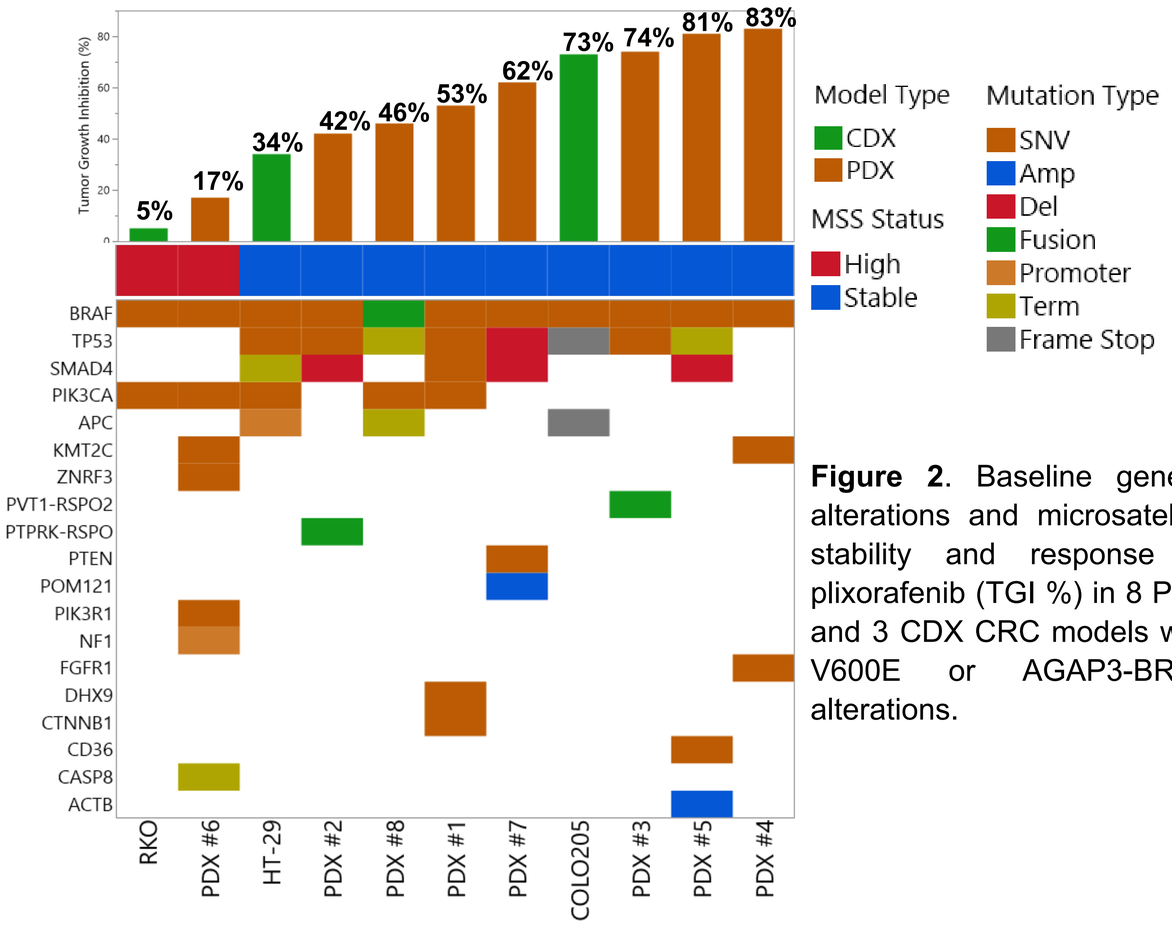
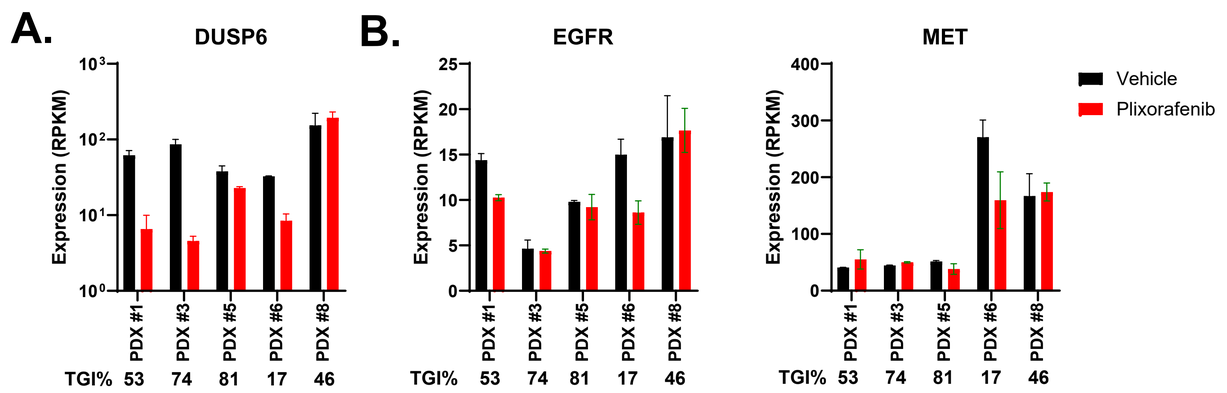


Figure 2. Baseline genetic alterations and microsatellite stability and response to plixorafenib (TGI %) in 8 PDX and 3 CDX CRC models with V600E or AGAP3-BRAF alterations.

High baseline EGFR/MET expression may predict less response to plixorafenib in PDX models



C. Top 5 GO and KEGG Pathways Associated with Response

Resistant to Plixorafenib		Sensitive to Plixorafenib	
Gene Ontology	**KEGG**	**Gene Ontology**	**KEGG**
Antiviral innate immune response	Human papillomavirus infection	Xenobiotic metabolic process	Porphyrin metabolism
Response to other organism	p53 signaling pathway	Pos regulation of transcription by RNA polymerase II	Metabolic pathways
Response to virus	Insulin resistance	B cell differentiation	Biosynthesis of cofactors
Apoptotic process	PI3K-Akt signaling pathway	Intraciliary transport	Retinol metabolism
Defense response to virus	Hypertrophic cardiomyopathy	Negative regulation of DNA-templated transcription	Inositol phosphate metabolism

Figure 3. Tumors were excised after 28 days of treatment with plixorafenib in 5 PDX models (4 $V600E^{mut}$, 1 AGAP3-BRAF fusion) and RNAseq was performed on tumors. (A) DUSP6 transcriptomic expression was measured as a readout for MAPK activity. (B) Transcriptomic expression of EGFR and MET in tumors after plixorafenib treatment or in vehicle group. (C) Pathway analysis of baseline transcriptomic expression that correlated with resistance or sensitivity to plixorafenib.

Combination with novel targeted therapies may mitigate intrinsic resistance in CRC

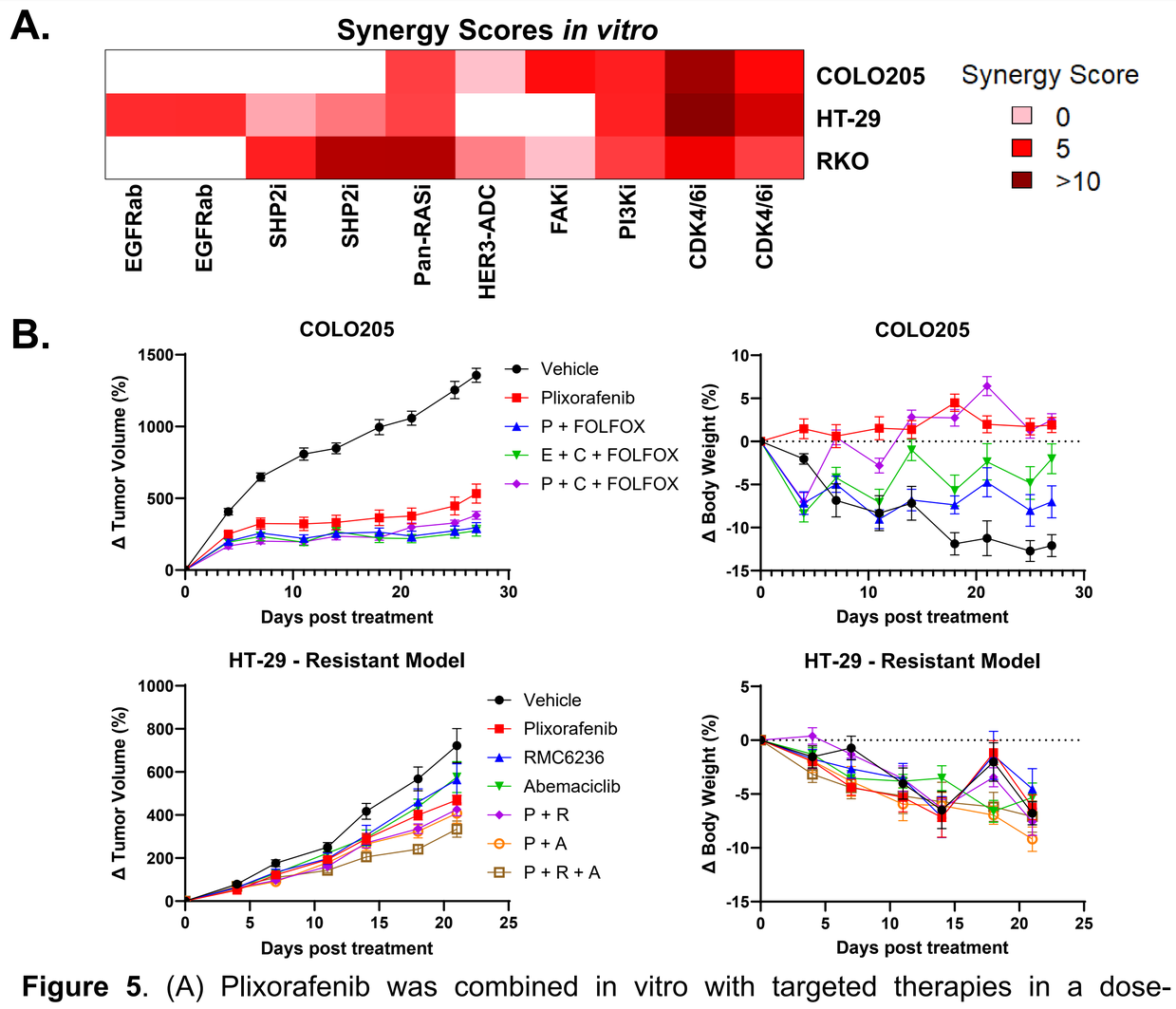


Figure 5. (A) Plixorafenib was combined in vitro with targeted therapies in a dose-dependent manner and synergy scores were calculated. (B) Mice were inoculated subcutaneously with CDX models and treated with plixorafenib combinations.

Conclusions

- Plixorafenib was active in 8 PDX and 3 CDX models of BRAF-altered CRC and demonstrated MAPK suppression in 5 models tested by RNAseq.
- PIK3CA mutations, particularly H1047R, and MSS-High status at baseline may predict lower response to plixorafenib, and supports rational combination with PI3K-AKT pathway inhibitors or I-O compounds.
- Lower sensitivity correlated with baseline expression in innate immunity, apoptosis/DNA repair, PI3K-AKT, and elevated MET or EGFR, whereas higher sensitivity was associated with metabolic programs and B cell differentiation.
- Consistent with other preclinical studies, plixorafenib treatment did not increase EGFR expression tested by RNAseq. This contrasts with 1st generation BRAFi, which increased EGFR expression and require addition of EGFR blockade for maximum MAPK suppression.
- Plixorafenib + FOLFOX combination was equivalent to encorafenib + cetuximab + FOLFOX triple combination in a head-to-head comparison, and addition of cetuximab did not increase tumor growth inhibition.
- Combination with novel targeted therapies, such as a pan-RASi and CDK4/6i, increase suppression of tumor growth in vitro and in vivo.