Plixorafenib (PLX8394/FORE8394) treatment leads to sustained tumor regression in preclinical models of BRAF-mutated primary and brain metastatic melanoma

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Introduction

- Plixorafenib is a novel, first-in-class paradox breaker that inhibits class 1, class 2, and fusion BRAF alterations.
- Mutations in the BRAF gene are found in 40-50% of melanoma patients, with V600E being the most common.
- While early generation BRAFi are available to treat V600-mutated primary and brain metastatic melanoma, paradoxical MAPK activity induced by these BRAFi results in poor tolerability and low durable response, particularly in patients with CNS metastasis.
- Plixorafenib is a selective, potent BRAFi that targets mutated BRAF monomers, BRAF homodimers, and BRAF-CRAF heterodimers without inducing RAF dimer formation.
- Unlike other BRAF inhibitors, plixorafenib evades paradoxical MAPK activation by disrupting BRAF dimer formation and reduces side effects and resistance associated with early generation BRAFi.
- In a Phase 1/2a study (NCT02012231), anti-tumor activity of plixorafenib was seen across multiple solid tumors, including BRAF-altered melanoma and primary CNS tumors (66.7% ORR, mDOR of 13.9 months).
- Thus, we hypothesized plixorafenib would be effective for patients with BRAF^{mut} melanoma with CNS metastasis.

Plixorafenib monotherapy is effective against subcutaneous model of V600E-mutated melanoma

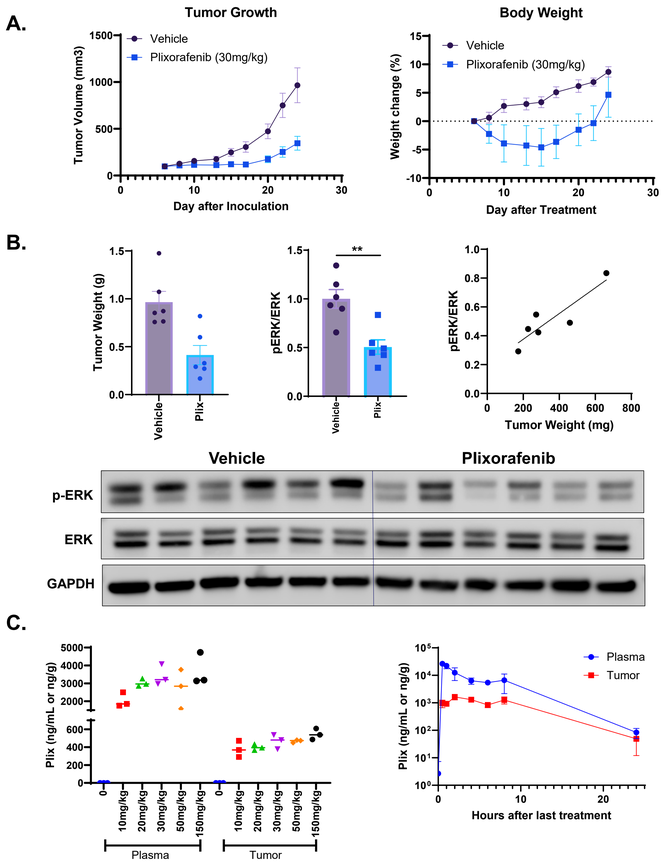


Figure 1. Mice were inoculated subcutaneously with A375 cell line (BRAF^{V600E}) and treated with plixorafenib at corresponding dosage for 21 days (A-B) or 5 days (C). **(A)** Tumor growth and body weight were measured throughout treatment. **(B)** At the end of treatment, tumor was excised, weighed, and measured for downstream MAPK signaling (pERK) by western blot. **(C)** Pharmacokinetics of plixorafenib were measured in the plasma and intra-tumor in the first 24 hours after the last dose.

Plixorafenib is effective in model of V600E melanoma brain metastasis

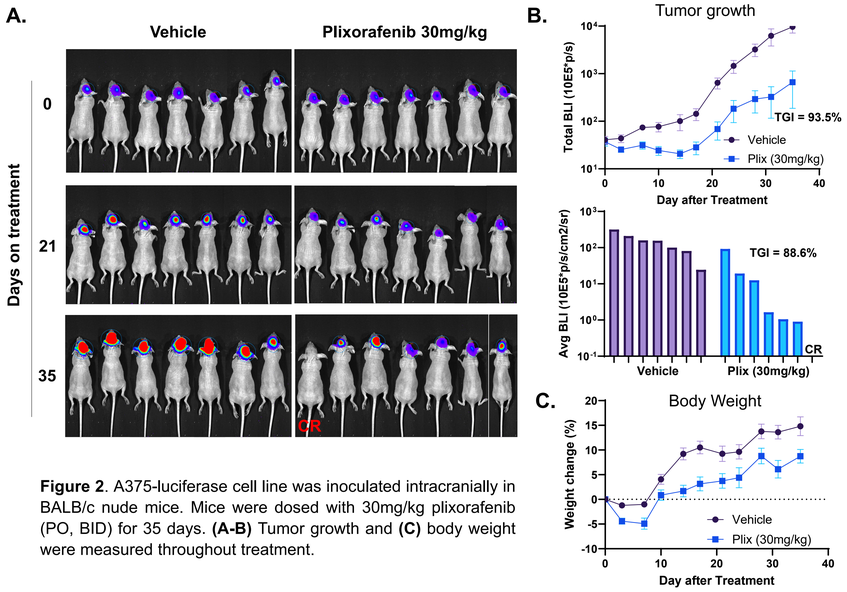


Figure 2. A375-luciferase cell line was inoculated intracranially in BALB/c nude mice. Mice were dosed with 30mg/kg plixorafenib (PO, BID) for 35 days. **(A-B)** Tumor growth and **(C)** body weight were measured throughout treatment.

Intra-tumor immune dysregulation after treatment with plixorafenib in brain metastasis model

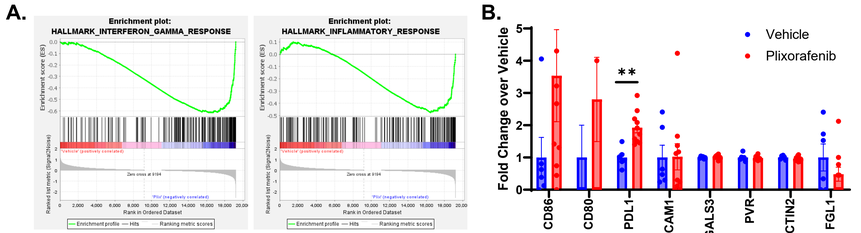


Figure 3. A375-luciferase cell line was inoculated intracranially and dosed twice daily with plixorafenib for 35 days. Intracranial tumors were excised and RNAseq was performed on tumors. Gene expression and pathway analysis were analyzed by Gene Set Enrichment Analysis (GSEA). **(A)** Enrichment plots of the top dysregulated Hallmark pathways after plixorafenib treatment, Interferon Response and Inflammatory Response. **(B)** Transcriptomic expression of immune checkpoint ligands in tumors after plixorafenib treatment or in vehicle group.

Transcriptomic changes in sensitive and resistant intracranial tumors

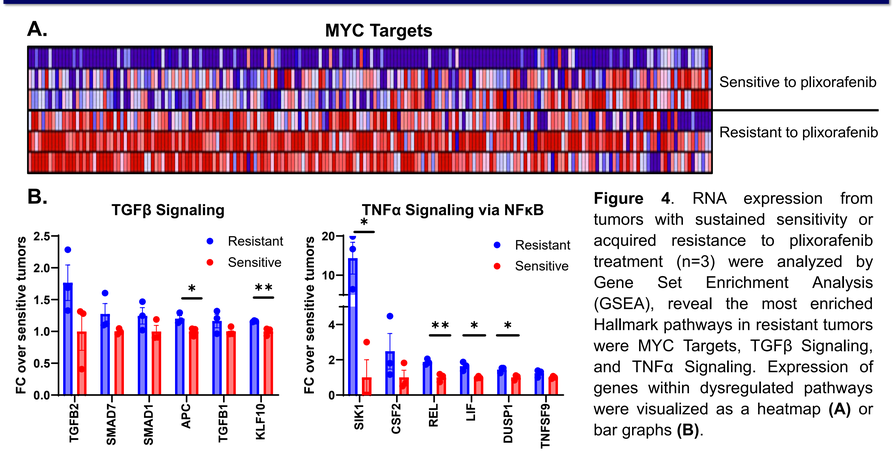
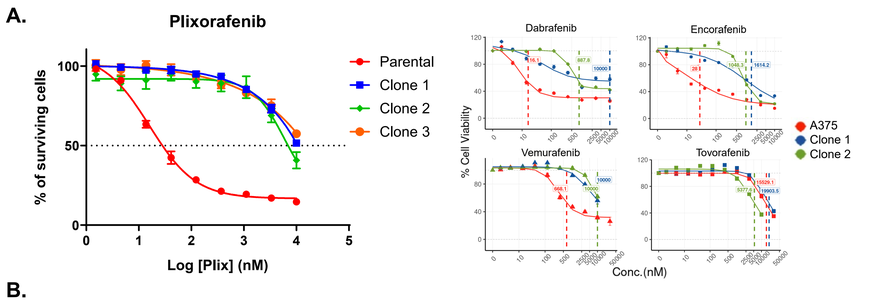


Figure 4. RNA expression from tumors with sustained sensitivity or acquired resistance to plixorafenib treatment (n=3) were analyzed by Gene Set Enrichment Analysis (GSEA), reveal the most enriched Hallmark pathways in resistant tumors were MYC Targets, TGFβ Signaling, and TNFα Signaling. Expression of genes within dysregulated pathways were visualized as a heatmap **(A)** or bar graphs **(B)**.

Plixorafenib maintains MAPK pathway suppression in plixorafenib-resistant melanoma cell lines



C.

Top 5 Hallmark Pathways Dysregulated in Plixorafenib-resistant clones

Clone 1	Clone 2	Clone 3
E2F Targets	E2F Targets	E2F Targets
Xenobiotic Metabolism	Complement	TNFa Signaling
P53 Pathway	G2M Checkpoint	Complement
Epithelial mesenchymal Transition	Xenobiotic Metabolism	KRAS Signaling Up
Allograft Rejection	Angiogenesis	Xenobiotic Metabolism

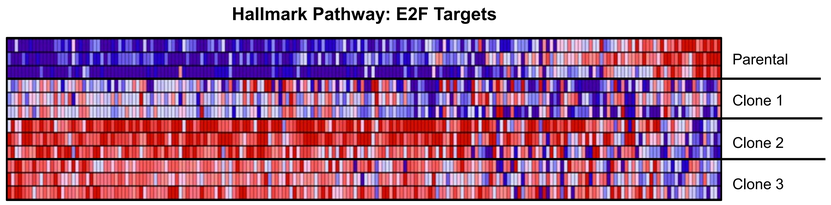


Figure 5. **(A)** In three plixorafenib-resistant A375 cell lines, cell viability was measured by CellTiterGlo after treatment with plixorafenib or other approved BRAFi. **(B)** Downstream MAPK activation (pERK) was measured by western blot. **(C)** RNAseq was performed on plixorafenib-resistant A375 cell lines, and gene expression was analyzed by Gene Set Enrichment Analysis, revealing the top dysregulated pathways in plixorafenib resistance.

Conclusions

- Plixorafenib was effective in both subcutaneous and intracranial model of BRAF^{V600E} mutated melanoma, with 71.4% and 88.6% tumor growth inhibition compared to vehicle group, respectively. In the brain metastatic model, one achieved histological complete response (n=7).
- Plixorafenib was well tolerated in both models, as no significant weight loss was observed throughout treatment.
- RNAseq from intracranial tumors at the end of treatment showed an increase in interferon and inflammatory responses, including the key immune checkpoint ligand PDL1, due to plixorafenib.
- Transcriptomics in CNS tumors with sustained tumor growth inhibition and those refractory to treatment were compared, revealing an enrichment in the Hallmark gene sets, MYC targets, TGFβ Signaling, and TNFα Signaling in resistant tumors.
- In three plixorafenib-resistant A375 cell lines generated in vitro, plixorafenib suppressed pERK activity in all three resistant clones despite limited activity on cell growth. RNAseq performed in parental and resistant cells showed an upregulation in the E2F targets and p53 signaling.
- These data suggest a dysregulation in cell cycling and DNA damage response may drive resistance to plixorafenib in melanoma, further supporting a differentiated mechanism of action and resistance for plixorafenib compared to early generation BRAFi.