Circulating tumor DNA analysis in patients with BRAF-mutated advanced unresectable solid tumors treated with plixorafenib (FORE8394/PLX8394) in Phase 1/2a study

#3248

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Introduction

- Plixorafenib is a selective, potent BRAFi that targets mutated BRAF monomers, BRAF homodimers, and BRAF-CRAF heterodimers without inducing RAF dimer formation[1].
- Plixorafenib actively inhibits class 1, class 2, and fusion BRAF alterations.
- Unlike early generation BRAF inhibitors, plixorafenib evades paradoxical MAPK activation by disrupting BRAF dimer formation and reduces toxicities and resistance associated with these BRAFi[1].
- A phase 1/2a dose escalation study was conducted in patients with advanced, unresectable solid tumors for safety and efficacy of plixorafenib. Results were previously presented at ASCO 2023[2-3].
- A total of 211 plasma samples were collected from 71 patients prior to treatment, throughout treatment, and at disease progression, and subsequently tested for circulating tumor DNA (ctDNA) using PredicineCare, a plasma ctDNA profiling assay (Predicine Inc), that detects single nucleotide variants (SNVs), insertions/deletions (INDELs), copy number variants (CNVs), and fusions in plasma samples down to 0.05% allele frequency.

Patient/Sample Characteristics	N (%)
Tumor Types	**No. of patients**
Papillary Thyroid Carcinoma (PTC)	15 (21.1)
Colorectal	14 (19.7)
Melanoma	10 (14.1)
Pancreas	7 (9.9)
Ovarian/Fallopian	6 (8.4)
Anaplastic Thyroid Carcinoma (ATC)	4 (5.6)
Medullary Thyroid Carcinoma (MTC)	4 (5.6)
Biliary	2 (2.8)
Small Bowel	2 (2.8)
Adrenal Gland	1 (1.4)
Angiosarcoma	1 (1.4)
Endometrium	1 (1.4)
Low grade glioma	1 (1.4)
Non-small cell lung cancer (NSCLC)	1 (1.4)
Prostate	1 (1.4)
Sarcoma	1 (1.4)
Alterations	**No. of patients**
All BRAF alterations	65 (91.5)
Class I	41 (57.7)
Class II SNVs	11 (15.5)
Fusions	10 (14.1)
Class III	1 (1.4)
Amplifications or Deletions	2 (2.8)
BRAF WT	2 (2.8)
KRAS mutations	4 (5.6)
Plasma Collection Timepoints	**No. of samples**
Baseline (C1D1)	66
C2D1	55
Other	63
End of Treatment	27
Total samples	211

1. Yao *Nature Medicine* 2019
2. Sherman *ASCO annual meeting 2023*
3. De La Fuente *ASCO annual meeting 2023*
4. Long *Nature Communications* 2014
5. Johnson *Eur Journal of Cancer* 2015
6. Ahronian *Cancer Discovery* 2015

Mutations from plasma ctDNA have high concordance with biopsy tissue NGS results

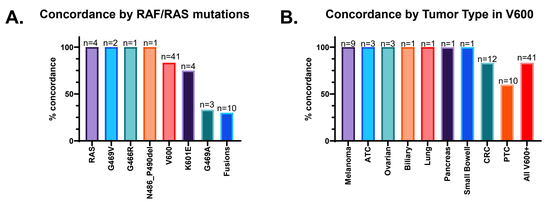

Figure 1. Concordance of targeted gene alterations in subjects with matched plasma ctDNA by PredicineCare and tissue NGS results from local lab tests at baseline and categorized by genetic alteration (A) and tumor type (B).

BRAF VAF% decreases following plixorafenib treatment, and is validated by pERK activity in paired tissue biopsy

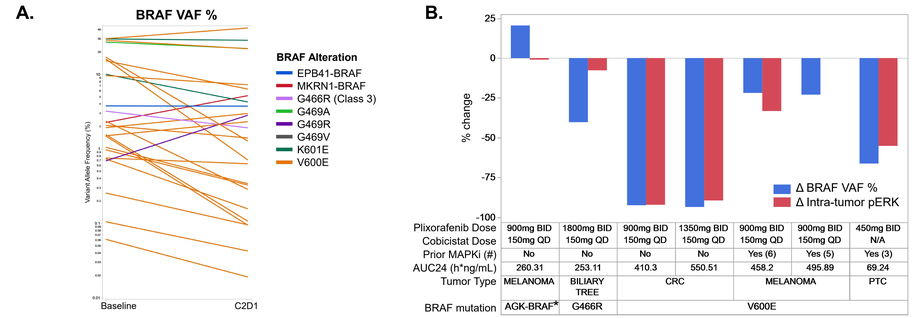

Figure 2. (A) BRAF V600E and non-V600 Variant Allele Frequency (VAF) % was measured at baseline and after one cycle of treatment, showing a decrease in BRAF VAF% in 17/20 V600E patients and 4/7 non-V600E patients. (B) For 7 patients with available paired tumor biopsy, pERK activity was measured at baseline and C1D8, and compared to BRAF VAF% changes from baseline to cycle 2 in ctDNA. *For AGK-BRAF patient, available plasma sample at C3D1 was used instead of C2D1.

Early declines in V600E VAF% may predict response to plixorafenib

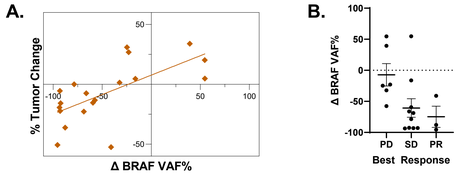

Figure 3. The percentage change in V600E VAF in plasma ctDNA from pre-treatment to cycle 2 was correlated to the greatest tumor volume change (A) or stratified by best overall response (B).

Changes in gene mutations and tumor fraction from ctDNA correspond with tumor volume across indications and BRAF mutations

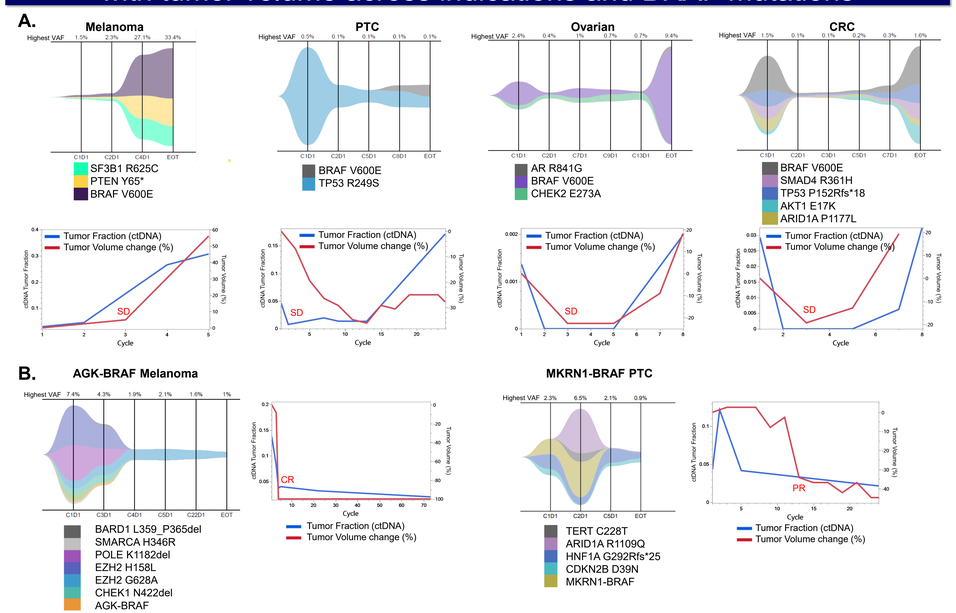

Figure 4. Representative plots of longitudinal analysis of ctDNA VAFs are compared to ctDNA tumor fraction and tumor volume in V600E-mutated (A) or BRAF fusion (B) patients across multiple indications, including melanoma, PTC, ovarian, and CRC.

No emerging MAPK mutations after plixorafenib treatment

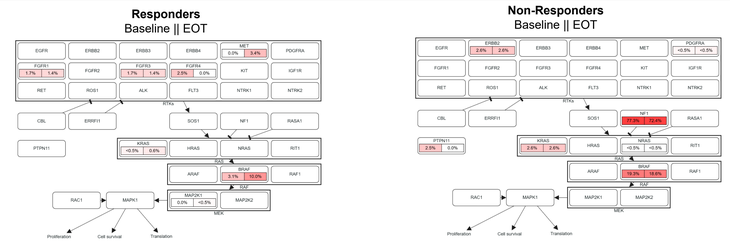

Figure 5. ctDNA gene mutations at baseline and end of treatment (EOT) were quantified in responders (CR, PR, SD) and non-responders (PD). Mutations were mapped to the MAPK pathway and visualized using PathwayMapper.

Intrinsic mechanisms of resistance drive lack of response in CRC

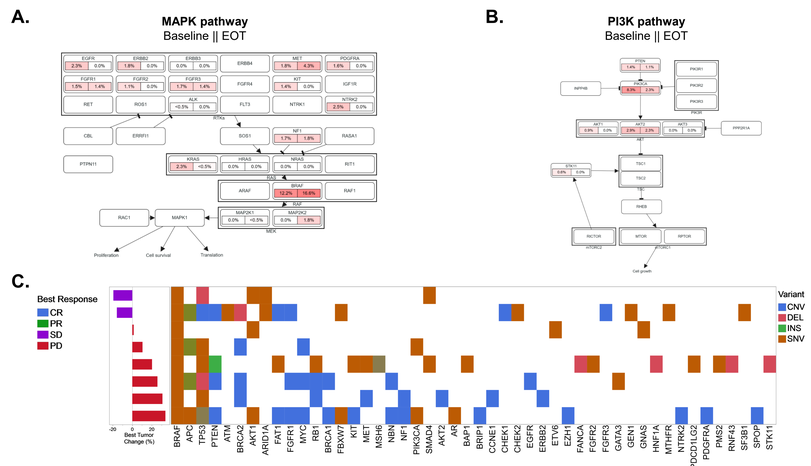

Figure 6. In CRC patients, mutations from ctDNA at baseline and the end of treatment (EOT) were quantified and mapped to the oncogenic pathways, MAPK (A) or PI3K signaling (B). (C) Oncoprint of ctDNA genetic mutations prior to plixorafenib treatment in V600E-mutated CRC patients (n=8), revealing the prevalence of intrinsic mechanisms of resistance in receptor tyrosine kinases and PI3K/AKT pathway.

Conclusions

- Plasma ctDNA results have high concordance with biopsy tissue NGS at baseline across tumor types and mutations, although some gaps appear in PTC patients and BRAF fusion alterations.
- Declines of V600E VAF% were observed in 85% subjects after one cycle of plixorafenib treatment. In patients with available paired tumor biopsies, decreases in pERK validated ctDNA results. VAFs in Class 2 and fusion also decreased, although responses in fusion VAFs may take several cycles to take effect. This data supports differentiated plixorafenib activity from early generation BRAFi.
- In V600E-mutated patients, early changes in V600E VAF% may predict response to plixorafenib, as responders had larger decreases in VAF% from baseline to cycle 2.
- In longitudinal samples, changes in ctDNA correspond to tumor size across tumor types, suggesting that ctDNA may be a surrogate marker for monitoring disease.
- Compared to acquired mutations driving resistance to early generation BRAFi's[4-6], no new mutations in MAPK pathway genes were found following plixorafenib treatment, supporting the dimer breaker property of plixorafenib.
- In non-responders, heterogeneity of genomic profile, co-occurrent RAS, MAPK-associated or NF1 mutations were seen, including melanoma pts who all received prior MAPKi therapies.
- In CRC, multiple driver mutations were found at baseline associated with receptor tyrosine kinase activation, confirming resistance pathways and supporting clinical utility of combination.