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RESEARCH

Phase II study of MEK inhibitor trametinib alone and in combination with AKT inhibitor GSK2141795/uprosertib in patients with metastatic triple negative breast cancer

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Abstract

Purpose While MEK inhibitors demonstrated activity in metastatic triple negative breast cancer (mTNBC) preclinical studies, preclinical, and clinical studies implicate rapid development of resistance limiting clinical benefit. The purpose of this study was to determine response rate for Trametinib alone and in combination with Uprosertib in patients with mTNBC previously treated with chemotherapy.

Methods This was an open-label, two-part, phase II, single-arm, multicenter study. Patients first received Trametinib monotherapy (2 mg daily; Part I) then at progression transitioned to Trametinib (1.5 mg) plus Uprosertib (50 mg; Part II).

Results Between October 2013 and January 2017, 37 patients were enrolled to Part I. Subsequently, 19 patients entered Part II. Of the 37 patients receiving Trametinib monotherapy, 2 patients achieved partial response (PR) for an ORR of 5.4% (2/37) and an additional 6/37 (16.2%) achieved stable disease (SD). The clinical benefit rate (PR+SD) for patients receiving monotherapy was 21.6% (8/37). Of the 19 patients in Part II, 3 patients achieved PR for an ORR to Part II of 15.8% (3/19) and an additional 3 achieved SD. Median progression-free survival (PFS) was 7.7 weeks for Part I and 7.8 weeks for Part II. Circulating tumor DNA (ctDNA) clearance at C2D1 of Trametinib monotherapy was associated with improved PFS and overall survival.

Conclusion In patients with mTNBC, Trametinib monotherapy demonstrated limited efficacy and addition of Uprosertib was associated with numerically greater objective responses but no difference in PFS. Translational analyses suggest ctDNA clearance as a potential early biomarker of response.

Keywords Triple negative breast cancer · Tyrosine kinase inhibitors · MEK inhibition

Introduction

Breast cancer (BC) remains the most diagnosed cancer in women with over 300,000 new cases estimated in the Unites States in 2023 [1]. Triple negative breast cancers (TNBCs) constitute about 15–20% of BCs but is the most aggressive subtype with distant recurrence rates and mortality rates higher than those seen in other types [2, 3]. TNBC is characterized on standard pathologic evaluation by lack of immunohistochemistry expression (IHC) expression of estrogen receptor (ER), progesterone receptor (PR) and HER-2 protein [2, 4, 5]. Unlike ER positive and HER2-over-expressing BCs, while targeted therapy regimens exist for the treatment of TNBC their use is limited to specific subsets of patients; as such, chemotherapy remains the standard of care. TNBC's lack of responsiveness to standard treatment regimens represents a clinical challenge as about 80% of patients do not experience a complete response to treatment with chemotherapy; additionally, recurrence and metastasis rates after treatment remain high [6]. However, immunotherapies and targeted agents in combination with traditional

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chemotherapy are being used to treat patients with both metastatic TNBC (mTNBC) and non-metastatic TNBC [7–9].

The majority (71%) of TNBC are of the basal-like phenotype [5]. Preclinical data demonstrates activation of RAS/RAF/MEK/ERK pathway in basal-like breast cancer (BLBC) leads to chemotherapy resistance [10]. This MAP kinase pathway regulates essential processes involving proliferation and tumor cell survival [11]. Furthermore, activation of the pathway has been demonstrated in TNBC patients with residual disease after neoadjuvant cytotoxic therapy [12]. Hoeflich et al. demonstrated treatment with a MEK-targeted drug caused reduced tumor growth in BLBC models [10]. However, even in patients who initially respond, mechanisms of MEK inhibitor (MEKi) resistance can arise reducing the benefit of these agents [13]. One proposed mechanism of intrinsic resistance to MEK inhibition is through the ERK-independent PI3K/AKT pathway. In BLBC models, blockade of both RAS/RAF/MEK/ERK and PI3K/AKT signaling synergizes to overcome resistance to MEK inhibition [10].

Trametinib is a selective, allosteric inhibitor of MEK1/MEK2 activation and kinase activity initially approved for the treatment of BRAF V600E/K mutant melanoma which is now being studied in a variety of other malignancies including serous ovarian cancers and RAS mutated AML [14–18]. Uprosertib is a reversible pan-AKT inhibitor (AKTi) which acts on AKT1, AKT2, and AKT3 [19]. Here we report the results of a two-part multicenter phase II study designed to evaluate the clinical efficacy of Trametinib monotherapy and Trametinib in combination with Uprosertib in patients with mTNBC previously treated with chemotherapy.

Methods

Patient selection

Between October 2013 and January 2017, 37 patients were enrolled in Part I of the study. Key eligibility criteria included patients with invasive mTNBC negative for the estrogen receptor (ER), progesterone receptor (PR), and HER2 by institutional guidelines, 18 years of age or older, Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, life expectancy of greater than three months, adequate organ function, and exposure to between 1 and 3 prior lines of chemotherapy regimens for the treatment of mTNBC. Asymptomatic patients with brain metastasis previously treated with surgery or stereotactic radiosurgery were allowed. Prior investigational drug therapy, a history of predisposing factors to retinal vein occlusion (RVO) or central serous retinopathy (CSR), uncontrolled hypothyroidism, and symptomatic or progressive brain metastases were exclusion criteria. All patients provided informed consent, and the protocol was approved by local ethics committees.

Study design

This was a single arm, multicenter, phase II study to evaluate the clinical activity of Trametinib and Trametinib in combination with Uprosertib in patients with mTNBC (Fig. 1). Patients were enrolled onto Part I where they received 2mg of oral Trametinib daily for a 28-day cycle. At the time of progression, patients were subsequently enrolled in Part II and then received 50mg of oral Uprosertib daily in addition to 1.5mg of oral Trametinib daily on a 28-day cycle. Safety assessments and laboratory tests were performed pre-dose, on day 28, and every 4 weeks thereafter. ECGs were obtained pre-dose, on day 15, and every 4 weeks after the first dose. An echocardiogram was performed pre-dose and every 12 weeks thereafter (or more frequently, if clinically indicated). Labs were obtained on day 1 of each cycle. Plasma samples were collected on day 1 of cycle 2 (C2D1) and at progression. Mandatory research biopsies were obtained pre-dose and at time of progression on Part I. Optional research biopsies were obtained at time of progression on Part 2. CT scans were obtained at baseline and after every 2 cycles, or when patients developed clinical signs concerning for disease progression. Disease assessment was performed at baseline and every 8 weeks according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST) [20]. The severity of toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0 [21].

Correlative studies

Core biopsies were obtained from each patient for correlative studies, including 4 samples that underwent formalin fixation and paraffin embedding as well as serial blood draws which were processed to component plasma and peripheral blood mononuclear cells (Fig. 1A).

Transcriptome

Five-micron sections used for RNA extraction were stained with H&E for quality control from each tissue block. RNA was purified from 5 µm thick tissue sections containing greater than 80% tumor using High Pure FFPE RNA Micro Kit (Roche) according to manufacturer's instructions. A minimum of 4 sections per sample were required. Affymetrix arrays were performed to determine transcriptional profiles of patient samples using Affymetrix GeneChip® Human Transcriptome Array 2.0 (HTA). Raw HTA data was normalized at the gene level using Robust Multi-Chip Averaging (RMA) algorithm as implemented in the

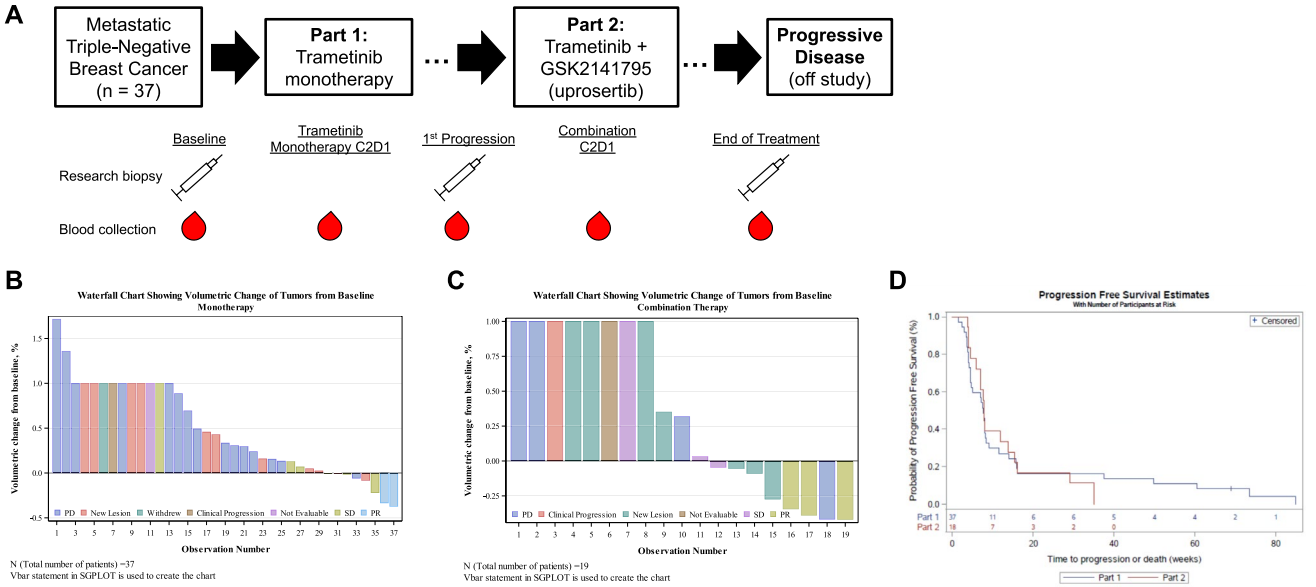

Fig. 1 Trial design and clinical outcomes. **A** clinical trial and correlative sample collection schema. On Part I, patients received trametinib monotherapy 2 mg oral daily. At time of progression, a tumor biopsy was obtained, and the patient was placed on Part 2. On Part 2, the patient received combination therapy with trametinib 1.5 mg oral daily and GSK2141795/uprosertib 50 mg oral daily. An optional tumor biopsy was obtained at time of disease progression on Part 2. **B**, **C** best percentage change from baseline in target lesions for Part I monotherapy (**B**) and combination therapy (**C**). **D** Kaplan–Meier estimated progression free survival for all patients. (Blue) Part 1. The median PFS for patients receiving monotherapy was 7.7 weeks (Red) Part 2. Median PFS for combination therapy was 7.8 weeks

Affymetrix® Expression Console™ Software 1.3 and then mapped the probe set identifiers to gene symbols based on the annotation file downloaded within the Expression Console application. Probe sets mapped to multiple genes were eliminated. When multiple probe sets were mapped to the same gene symbol, the probe set with largest interquartile range was kept. 125 previously published signature scores were calculated as previously published [22] and Gene Set Enrichment analysis for the Hallmark signatures was completed comparing available pre-/post-trametinib monotherapy and responders versus non-responders [23].

Circulating tumor DNA analyses

Venous blood samples were collected from enrolled patients in EDTA (BD) or Cell-Free DNA BCT (Streck) tubes. Blood processing to component elements, cell-free DNA extraction from plasma, and DNA quantification was performed as described previously [24]. The Kapa HyperPrep kit with custom adapters (IDT) was used to construct a library of cell-free DNA (cfDNA). 5–50 ng of cfDNA input was used for ultra low-pass whole-genome sequencing (ULP-WGS). Libraries were pooled (2 uL of each × 96 per pool) and sequenced as previously detailed to average genome-wide fold coverage of 0.1X. Segment copy number and TFx were derived via ichorCNA [24]. Samples were excluded due to poor quality data if the median absolute deviation of the copy ratios between adjacent bins was greater than 0.20.

Statistical analysis

The primary end point of this study was objective response rate (ORR) to trametinib monotherapy defined as the proportion of patients who have had a partial response (PR) or complete response (CR) within the first 6 months after initiation of therapy with trametinib. For monotherapy the null hypothesis was an ORR of $\leq 5\%$; the alternative hypothesis was an ORR of $\geq 20\%$. With 90% power and a Type 1 error rate of 10%, the interim analysis for this design required at least one of the first 12 evaluable patients enrolled had an objective response to treatment. For combination therapy, the null hypothesis was an ORR $\leq 5\%$. With 90% power and a type 1 error rate of 10%, this Fleming two-stage design had an interim analysis after the first 16 evaluable patients were accrued. Because at least one patient had a clinical response out of the first 16 enrolled patients, accrual continued to a total of 19 patients. ORR were estimated independently for monotherapy and combination therapy and corresponding 95% binomial confidence intervals were generated. PFS was summarized using Kaplan–Meier plots and log-rank test for comparisons related to ctDNA.

Results

Patients

Between October 2013 and January 2017, 37 patients were enrolled across 8 sites to Part I and, subsequently, 19 of these patients entered Part II (Fig. 1A.). Patients had a median age of 57 (range 35–71) and most patients were Caucasian (76%) with 11% Asian and 8% Black/African American (Table 1.). Most patients (23/37; 62%) had visceral disease and three prior lines of therapy for mTNBC (20/37; 54%). The study closed before the proposed accrual of up to 41 patients due to a short supply of Trametinib. By the clinical cut-off date of January 23, 2017, all 37 patients had completed or discontinued study treatment. At the clinical cutoff date, 25 patients had died of disease (25/37; 68%), 9 patients were off-study and alive with disease (9/27; 24%), and 3 patients were in study follow-up (3/37; 8%). The duration of monotherapy was 2 cycles (1–21 cycles) and the median duration of combination therapy was 2 cycles (1–8 cycles).

Table 1 Baseline patient characteristics

Characteristic	No. (%) n = 37
Age (years)	
Median (range)	57 (35–71)
Race	
Caucasian	28 (76%)
Asian	4 (11%)
Black/African-American	3 (8%)
Unknown	2 (5%)
ECOG performance status	
0	19 (51%)
1	18 (49%)
Sites of metastatic disease	
Visceral	23 (62%)
Non-visceral only	14 (38%)
Number of prior therapies for metastatic TNBC	
1	9 (24%)
2	8 (22%)
3	20 (54%)

Clinical outcomes

The primary end point of this study was ORR to Trametinib monotherapy. Of the 37 patients on Trametinib monotherapy, 2 patients had a partial response (PR) for an ORR of 5.4% (2/37) (Table 2; Fig. 1B). An additional 6 patients (16.2%) had stable disease (SD) as best response with one patient having SD for 21 cycles of therapy. The clinical benefit rate (PR+SD) for patients receiving monotherapy was 21.6% (8/37). The duration of response for those who achieved a PR on monotherapy was 53.8 weeks (range 42.1–65.4) and 8 weeks (range 6.4–77.1) for those who achieved SD as best response.

Within the stepwise design of this study, response criteria were achieved in Part I to continue to enroll to Part II, and the study met interim analysis criteria of one objective response in the first 16 patients entering Part II to enroll 19 total patients in part II. As a secondary endpoint, the ORR to Part II was 15.8% (3/19). Of the 19 evaluable patients in Part II, 3 patients had a PR, including one patient who unintentionally received an increased dose of trametinib, and 3 patients achieved SD as best response (Table 2; Fig. 1C). The duration of response for those who achieved a PR on combination therapy was 15.4 weeks (range 12.9–28.3) and 7 weeks (range 2.6–8.43) for those who achieved SD.

The median PFS for the patients receiving Trametinib monotherapy (Part I) and Trametinib plus Uprosertib combination therapy (Part II) was 7.7 weeks and 7.8 weeks, respectively (Fig. 1D). The median overall survival (OS) was 34.1 weeks (range 4.9–144.0 weeks).

Treatment-related toxicity

All 37 patients received at least one dose of Trametinib. The most frequent treatment-related AEs with both monotherapy and combination therapy are listed in Table 3. There was one treatment-related death with monotherapy, characterized by diagnosis of osteomyelitis and severe sepsis shortly after starting the trial in a patient with baseline diabetes mellitus. There were no grade 4 toxicities. With monotherapy, across all grades the most common adverse events were fatigue (27%), elevated AST (27%), acneiform rash (27%) and nausea (22%). With combination therapy across all grades, the most common adverse events were diarrhea (84.2%) and oral

Table 2 Clinical efficacy

		Confirmed objective response rate (ORR)					Median progression-free survival (PFS)	
Part	n	PR	SD	PD	NE	ORR (%)	PFS (weeks)	Range
I	37	2	6	27	2	5.4	53.8	42.1–65.4
II	19	3[a]	3	12	1	15.8	15.4	12.9–28.3

PR partial response, *SD* stable disease, *PD* progressive disease, *NE* non-evaluable

[a]One patient received higher dose of trametinib on Part II

Table 3 Treatment-related adverse events occurring in ≥ 10% of patients, including all grade 3 toxicities

Adverse event	All grades No. (%)	Grade 2 No. (%)	Grade ≥ 3 No. (%)
Part I monotherapy (n = 37)			
Nausea	8 (21.6)	4 (10.8)	0 (0)
Fatigue	10 (27.0)	5 (13.5)	1 (2.7)
AST increased	10 (27.0)	0 (0)	1 (2.7)
Hypoalbuminemia	5 (13.5)	1 (2.7)	1 (2.7)
Edema limbs	7 (18.9)	3 (8.1)	1 (2.7)
Left ventricular systolic dysfunction	1 (2.7)	0 (0)	1 (2.7)
Thromboembolic event	1 (2.7)	0 (0)	1 (2.7)
Neutrophil count decreased	1 (2.7)	0 (0)	1 (2.7)
Mucosal infection	1 (2.7)	0 (0)	1 (2.7)
Generalized muscle weakness	1 (2.7)	0 (0)	1 (2.7)
Musculoskeletal and connective tissue disorder	1 (2.7)	0 (0)	1 (2.7)
Neoplasms benign, malignant and unspecified	1 (2.7)	0 (0)	1 (2.7)
Renal and urinary disorders	1 (2.7)	0 (0)	1 (2.7)
Rash acneiform	10 (27.0)	4 (10.8)	0 (0)
Part II combination therapy (n = 19)			
Anemia	4 (21)	2 (10.5)	0 (0)
Colitis	1 (5.3)	0 (0)	1 (5.3)
Enterocolitis	1 (5.3)	0 (0)	1 (5.3)
Diarrhea	16 (84.2)	3 (15.8)	6 (31.6)
Mucositis oral	7 (36.8)	4 (21.1)	0 (0)
Dehydration	3 (15.8)	2 (10.5)	1 (5.3)
Fatigue	5 (26.3)	3 (15.8)	0 (0)
Dyspnea	4 (21)	2 (10.5)	1 (5.3)
Left ventricular systolic dysfunction	1 (5.3)	0 (0)	1 (5.3)
Hypertension	4 (21)	1 (5.3)	1 (5.3)
Infections	3 (15.8)	1 (5.3)	1 (5.3)
Pruritus	2 (10.5)	2 (10.5)	0 (0)
Rash maculo-papular	2 (10.5)	2 (10.5)	0 (0)

mucositis (37%). In total, 31.6% of patients experienced ≥ 3 grade diarrhea. No cases of retinal vein occlusion were observed. 1 patient (3%) on monotherapy and 1 patient (5%) on combination therapy experienced left ventricular ejection fraction reduction. Overall, among 37 patients enrolled in the study 29/37 (78.4%) patients discontinued study treatment because of disease progression, 4/37 (10.8%; 2/37 on Part I and 2/37 on Part II) discontinued study treatment because of toxicity, 3/37 (8.1%) patients withdrew consent to pursue non-study treatments, and 1/37 (2.7%) patient remained on treatment until trametinib became unavailable.

Correlative analyses

Pre-treatment biopsies were obtained on 34/37 (92%) patients, Part I progression biopsy was obtained on 15/37 (41%) patients, and Part II progression biopsy was obtained on 2/37 (5.4%) patients. Blood for ctDNA analysis was collected on 31/37 (84%) patients, with samples available for analysis at C1D1 (34/37; 92%), C2D1 (22/37; 59%), Part I/trametinib monotherapy progression (15/37; 41%), C2D1 of Part II: combination treatment (14/19; 74%), and Part II combination treatment progression (18/19; 95%).

Paired pre-treatment and trametinib resistance/post-treatment transcriptome analyses

Transcriptome was evaluated via microarray on 14 pre-/post-trametinib monotherapy pairs, with diverse best ORR (PR n = 1; SD n = 3, PD n = 10). Three published RAS/ERK signatures were identified a priori for analyses based on the study: a Ras/Erk Activation signature [26], a Ras Activation signature, and a MAPK signature. (Fig. 2A). While there was a trend toward higher RAS-MAPK-ERK activation at progression relative to baseline, none of the three were statistically significant (Wilcoxon rank-sum). As an exploratory evaluation, Hallmark gene sets were evaluated between pre- vs. post-trametinib monotherapy pairs via Gene Set

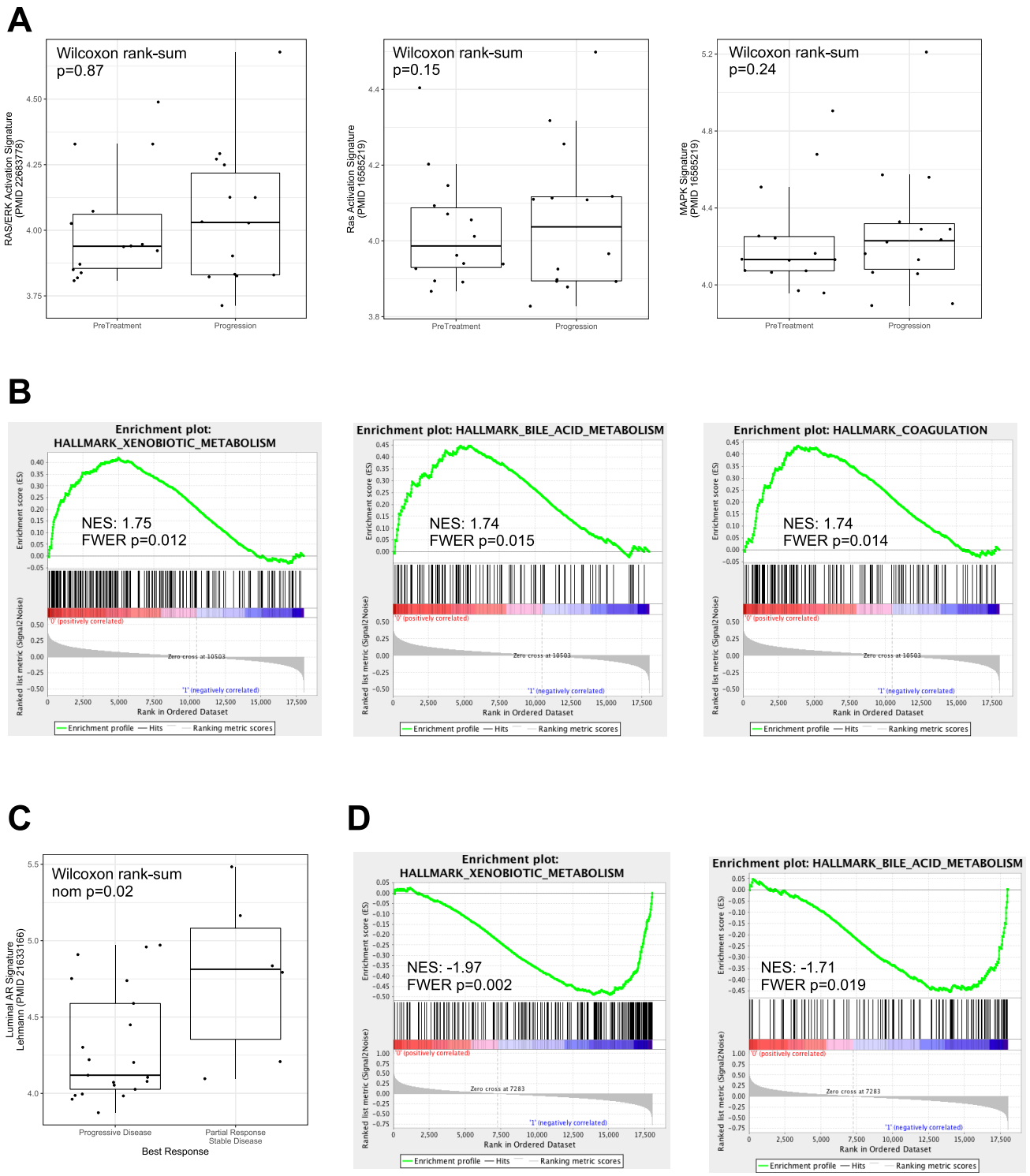

Fig. 2 Transcriptome analyses implicate metabolic processes after trametinib exposure. **A** microarray analysis of 14 pre-/post-trametinib monotherapy matched pairs for Ras/Erk [25, 26], Ras [48], and MAPK [48] activation signatures. **B** Gene Set Enrichment Analysis (GSEA) enrichment visualization of statistically significant (FWER $p < 0.05$) Hallmark gene sets comparing pre- vs. post-trametinib monotherapy pairs—xenobiotic metabolism, bile acid metabolism, and coagulation. **C** Microarray analysis of Lehmann androgen receptor [47] signature comparing PD vs PR + SD. **D** GSEA enrichment visualization of statistically significant (FWER $p < 0.05$) pre-treatment Hallmark gene sets comparing those with PR+SD vs. *PD* xenobiotic metabolism and bile acid metabolism. *NES* normalized enrichment score. *FWER* family-wise error rate. *Nom* nominal

Enrichment Analysis (GSEA) (Fig. 2B). Three Hallmark signatures were significant: xenobiotic metabolism (NES 1.75, FWER p = 0.012), bile acid metabolism (NES 1.74, FWER p = 0.015), and coagulation (NES 1.74, FWER p = 0.014). Top up- and down-regulated single genes by pre- vs. post-trametinib are provided in Supp Fig. 1. As a second exploratory analysis, we evaluated those patients with clinical benefit (PR+SD) versus those with progressive disease as best response (Fig. 2C, D). First, 125 published signatures [22] were evaluated and while no signature achieved statistical significance after controlling for multiple tests, the Lehmann androgen receptor (AR) signature was higher in patients with clinical benefit (nominal p = 0.02; Fig. 2C). GSEA analysis of Hallmark gene sets revealed that two signatures were higher in pre-treatment samples for those with PR/SD: xenobiotic metabolism (NES -1.97, FWER p = 0.002), bile acid metabolism (NES − 1.71, FWER p = 0.019) (Fig. 2D.). Top up- and down-regulated single genes by best response are provided in Supp Fig. 2.

Circulating tumor DNA analyses

There is growing data suggesting that ctDNA 'tumor fraction' (TFx) is prognostic, particularly among patients with mTNBC [27], and that early change of ctDNA TFx may be predictive of response to therapy [28–31]. Based on these data, we focused ctDNA analyses on early time points as prognostic (C1D1 alone) and early predictive (C1D1:C2D1). ctDNA was evaluable in 34 patients at C1D1 (tumor fraction (TFx) range 0–75.9%, median 5.5%). Baseline/C1D1 TFx was significantly lower in patients who achieved SD or PR as best response relative to those with PD (TFx range 0–11.9% vs. 0–75.9%, TFx median 3.8% vs. 13.7%, t-test p = 0.014; Fig. 3A). We then evaluated early change in ctDNA TFx (C1D1:C2D1) among 22 patients with evaluable ctDNA TFx at both time points (Fig. 3B). While the change from C1D1 to C2D1 was not significant among either those achieving SD+PR (Wilcoxon signed-rank p = 0.48) or those achieving PD as best response (Wilcoxon signed-rank p = 0.64), we noted six patients had ctDNA 'clearance' (TFx 0%) at C2D1. While the numbers were small, of these six, 3/6 (50%) had PR as best response, and an additional 2/6 (33%) had SD as best response, compared with PR in only 1/16 (6%) and SD in 4/16 (25%) of patients without ctDNA clearance at C2D1 (Fig. 3C). We then evaluated baseline TFx as well as early change in ctDNA TFx with patient outcomes, including PFS (Part I+Part II if enrolled) and OS (Fig. 3D–G). Using an established TFx threshold of 10% (TFx ≤ 10% versus > 10%), [27, 32] baseline TFx was significantly associated with both PFS (log-rank p = 0.042; Fig. 3D) and OS (log-rank p = 0.001; Fig. 3E), with TFx ≤ 10% demonstrating significantly improved outcomes. Further, early change in ctDNA C2D1 was predictive and prognostic, with significant association of clearance at C2D1 with improved PFS (log-rank p = 0.01; Fig. 3F) and OS (log-rank p = 0.01; Fig. 3G), though interpretation limited by small number of responders.

Discussion

TNBC treatment remains a therapeutic challenge as targeted therapy usage is limited to specific patient subsets in this aggressive subtype. Based on robust preclinical data implicating activation of the MEK/ERK pathway in mTNBC and activation of the PI3K/AKT pathway as a resistance mechanism to single agent MEKi, we sought to investigate usage of MEKi, Trametinib, alone and in combination with AKTi, GSK2141795/Uprosertib, in patients with mTNBC who had previously been treated with systemic chemotherapy. The ORR rate for Trametinib monotherapy was 5.4% and ORR to Trametinib plus Uprosertib combination therapy was 15.8%, suggesting that Trametinib does not appear to be highly active in this setting alone or in the tested combination. While a greater proportion of patients showed some clinical benefit (defined as PR+SD)—21.6% for Trametinib monotherapy and 31.5% for Trametinib plus Uprosertib combination therapy—the duration of response was limited and less than 8 weeks for both monotherapy and the combination. The study closed before proposed accrual of up to 41 patients due to short supply of Trametinib.

Trametinib is a potent MEKi initially approved for the treatment of BRAF V600E/K mutant melanoma and now approved for the treatment of other BRAF V600E mutation positive solid tumors in combination with Dabrafenib [33]. Trametinib has been found to directly act on the Kinase-Suppressor of Ras (KSR) at the MEK interface. This MEKi constitutes the backbone of numerous investigational combination therapies alongside drugs including Dabrafenib and also various immunotherapies [34–36]. Uprosertib, on the other hand, is a AKTi theorized to work in combination with Trametinib to overcome MEKi resistance [37]. This two drug combination has been studied in metastatic melanoma, endometrial carcinoma, and cervical cancer due to preclinical studies suggesting clinical benefit in a wide array of tumors [38–40].

While MEKis are a drug class theorized to profoundly impact the therapeutic landscape of numerous malignancies, MEKi resistance and adverse effects have limited their clinical efficacy. This remains true in mTNBC, as well, as exemplified by the sparsity of responders in our trial. Literature has previously reported numerous theories leading to MEKi resistance but structural analysis of Trametinib itself has identified that Kinase Suppressor of RAS (KSR) binding, which is vital in Trametinib binding to MEK, plays a role in developing MEKi resistance [18]. The study by Khan et al. reports on a novel MEKi in which the adaptive MEKi resistance associated with Trametinib,

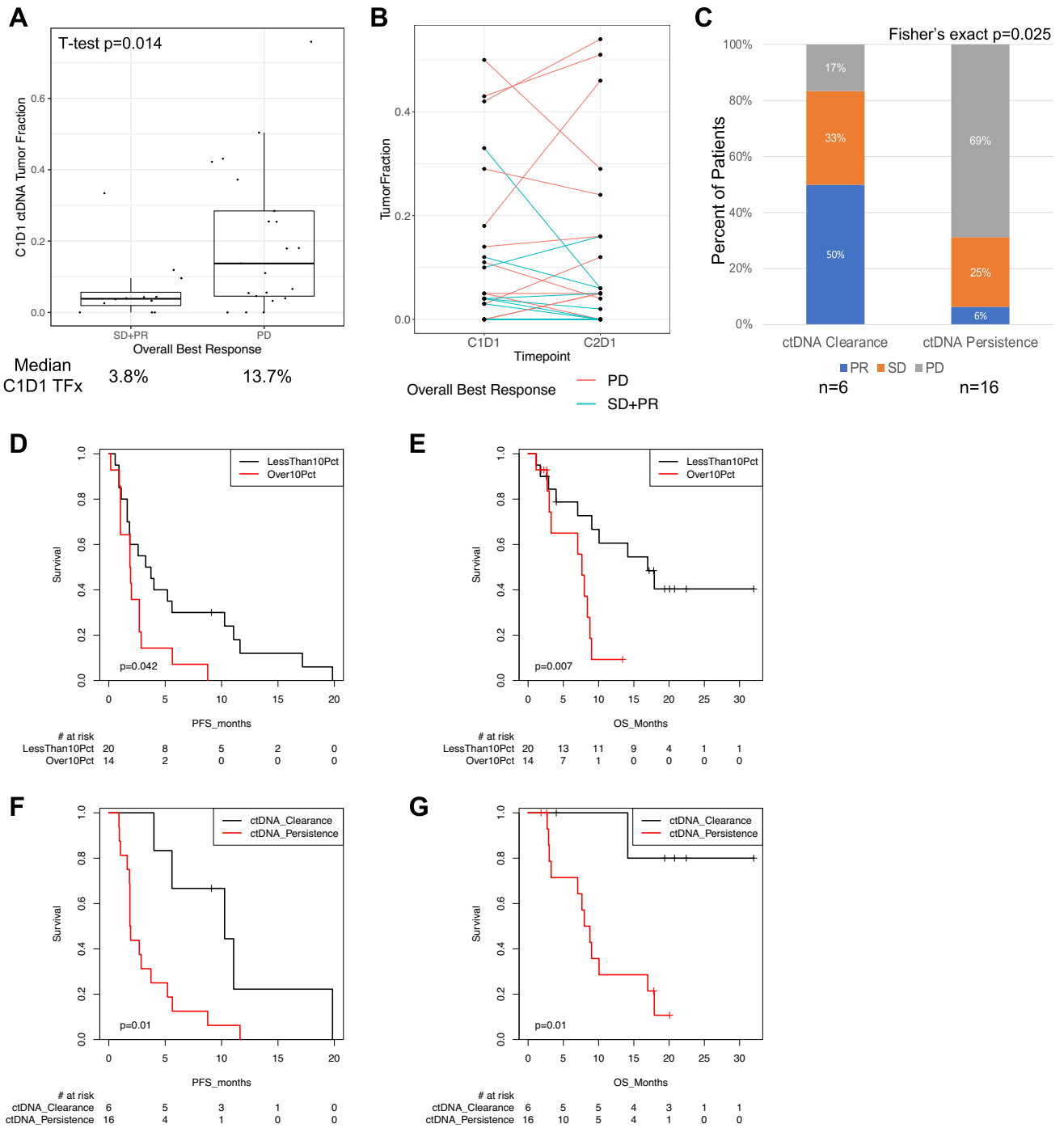

Fig. 3 Circulating tumor DNA analysis reveals decline in tumor fraction at C2D1 in most responders. **A** boxplot of baseline/C1D1 TFx in responders vs non-responders; **B** paired line plot of 22 patients with evaluable ctDNA TFx data at baseline and as C2D1 (responders = blue, non-responders = red); **C** bar chart of ctDNA clearance, defined as no detectable ctDNA (TFx = 0) at C2D1, versus ctDNA persistence, defined as detectable ctDNA (TFx > 0) at C2D1, by best response. **D–G** Kaplan–Meier curves of overall (Part I+Part II) progression-free survival (PFS) (**D**, **F**) and overall survival (**E**, **G**). Patients were stratified by baseline TFx ≤ 10% ('Less than 10%') versus TFx > 10% ('Over 10%') (**D**, **E**) or ctDNA clearance versus ctDNA persistence (**F**, **G**)

in particular, is reduced [18]. Thus, next-generation MEKis may constitute treatment modalities with improved clinical efficacy in TNBCs due to a reduction in MEKi resistance; this will theoretically lead to more clinical responders. Previously published literature appeared concordant with the findings of our study, whereby a narrow subset of patient with TNBC responded to trametinib alone and in combination with Uprosertib. In the 2020

Phase I study by Tolcher et al. studying both patients with metastatic melanoma and TNBC, the authors cited insufficient dosages and therefore drug concentrations as the limiting factor in attaining appropriate pathway inhibition [37]. The literature also reports on numerous clinical trials in metastatic melanoma, endometrial, and cervical cancer where the combination of Trametinib and Uprosertib did not lead to a significant clinical benefit and also had high levels of toxicity [38–40]. Furthermore, while MEK activation has strong preclinical data as a mediator of chemoresistance, it may be that combining Trametinib with other targeted therapies (such as Dabrafenib) [41] or mitochondrial protease caseinolytic protease agonist ONC201 [42]) or chemotherapy may be needed.

As this was a negative study, a major goal of the detailed correlative analyses was to potentially understand the reasons why Trametinib monotherapy and in combination with Uprosertib lacked efficacy. Zawistowski, et al. previously demonstrated significant transcriptional shifts in TNBC cell lines after treatment with Trametinib, including upregulation of receptor tyrosine kinases and evidence that *BRD4* inhibitors blunted the transcriptional response to Trametinib [43]. Further work by the same group suggested epigenomic modulation, not evaluated in this study [44]. While our pre- vs. post-Trametinib gene expression analyses did not identify RTKs, *BRD7* expression was upregulated suggesting bromodomain activation. In our transcriptome analysis, we identified a trend toward higher RAS-MAPK-ERK activation at progression relative to baseline, similar to preclinical models [10]. Interestingly, the significant Hallmark signatures upregulated in post- vs. pre-Trametinib and enriched in patients with clinical benefit vs. no benefit were metabolic: xenobiotic metabolism and bile acid metabolism.

While transcriptome analyses provided insight into transcriptional adaption, no clear predictive biomarker emerged. As an additional approach, we analyzed ctDNA TFx—a simple, accessible metric via minimally invasive blood sampling. In general, patients with clinical benefit (PR+SD) had lower ctDNA TFx at baseline, consistent with established data that lower TFx is associated with improved prognosis among mTNBC [27]. Perhaps more interesting are ctDNA dynamics as ctDNA clearance may be an early biomarker for therapy responsiveness especially in chemotherapies [45, 46]. Patients with undetectable ctDNA TFx at C2D1 (either non-shedders or detectable at C1D1 then cleared at C2D1), were significantly more likely to achieve clinical benefit. Further, we confirmed that baseline TFx was associated with prognosis, as other studies have shown [27, 32], while also demonstrating that early change was predictive. This reinforces the potential of early ctDNA dynamics as a potentially treatment agnostic minimally invasive biomarker [30].

This study has limitations. First, TNBC is a heterogeneous subtype of breast cancer. As our data suggested, it may only be certain subtypes of TNBC (e.g. luminal AR [47]) that sufficiently depend on MEK benefit from this treatment. Further, this is a heavily pretreated population with diverse prior treatments and clonal heterogeneity which may have limited the effectiveness of these targeted agents. Our correlative analyses, while detailed, were limited by the available samples despite efforts to collect extensive biopsy and blood at multiple time points. Additionally, analyses of association with outcomes were limited by the relatively few objective responses and short median duration on therapy.

Overall, this study did not show significant clinical benefit of either Trametinib monotherapy (MEKi) or combination therapy with Trametinib and Uprosertib (MEKi + AKTi). Future studies in the field could yet elucidate a subpopulation of patients with TNBC for whom treatment with MEKi and AKTi may have clinical efficacy.

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Data availability Bhuvaneswari Ramaswamy and Daniel G. Stover had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Declarations

Conflict of interest Authors have no conflicts of interest related to the current analyses.

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