Pilot trial of priming with oral TAK-228 and TAK-117 (PIKTOR) to increase DNA damage repair deficiency followed by cisplatin and nab paclitaxel in chemotherapy-pretreated metastatic triple negative breast cancer patients

Joyce O'Shaughnessy[1], Jessica Lang[2], William Hendricks[2], Esther San Roman Rodriguez[3], Priscilla Ontiveros[4], Rick Wenstrup[4], Victoria Zismann[2], Natalia Briones[2], Kendra Peck[1], Claudius Mueller[5], Virginia Espina[5], Maren K. Levin[3]

[1]Baylor University Medical Center, Texas Oncology, Dallas, TX; [2]The Translational Genomics Research Institute, Phoenix, AZ; [3]Baylor Scott & White Research Institute, Dallas, TX; [4]Epic Sciences, San Diego, CA; [5]George Mason University, Fairfax, VA

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INTRODUCTION

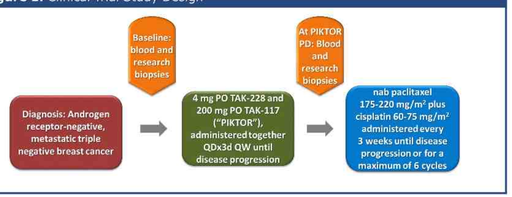
- Seventy to 80% of triple negative breast cancers (TNBC) are characterized by homologous recombination deficiency (HRD) and high proliferation.[1]
- HRD leads to upregulation of the activity of the non-homologous end joining (NHEJ) error-prone pathway that repairs DNA double strand breaks, a process required for TNBC survival.[2]
- We investigated the combination of TAK-228 and TAK-117 (PIKTOR), investigational oral TORC1/2 and PI3Kα selective inhibitors, respectively, to evaluate whether PIKTOR can increase genomic instability (GI) and increase DNA damage repair deficiency (DDRD), leading to increased sensitivity to DNA damaging chemotherapy and to checkpoint inhibitor therapy in metastatic (met)TNBC pts.[3]
- Primary endpoints were objective response rate with cis/nab pac following PIKTOR, and safety.

PATIENTS AND METHODS

Clinical Trial

- Following IRB-approved informed consent, 10 pts with androgen receptor-negative metastatic TNBC were enrolled, with key eligibility criteria: no more than 3 prior chemotherapy regimens for metastatic disease; ECOG PS 0-2; breast, chest wall, LN, pulmonary or hepatic metastatic disease amenable to core needle biopsy.
- Pts received 4 mg TAK-228 PO and 200 mg TAK-117 PO both QDx3d on, followed by 4 days off weekly until disease progression (PD), followed by cisplatin (cis) 75 mg/m^2 plus nab paclitaxel (nab pac) 175-220 mg/m^2 IV every 3 weeks until progression of disease (PD) or for a maximum of 6 cycles (**Figure 1**). Pts with benefit from cis/nab pac were then treated with pembrolizumab (pembro).
- Blood samples and research biopsies of metastatic lesions were collected prior to PIKTOR and at progression on PIKTOR (**Figure 1**).

Figure 1. Clinical Trial Study Design



Next Generation Sequencing

- Sequencing: Whole Exome Sequencing (WES) and RNA sequencing were performed on PIKTOR pre-treatment (Pre) and PIKTOR post-progression (Post) fresh-frozen biopsies. DNA and RNA were extracted with Qiagen DNeasy and RNeasy kits. All samples sequenced on NovaSeq6000, Paired-end x 100bp.
  - WES: Custom Agilent SureSelect kit (captures exonic regions and common structural variations in cancer).
  - RNA-seq: KAPA Stranded total RNA with ribo-depletion
- Analysis: QC, alignment, SNV calling, CN calling, and RNA quantification were performed using TGen's Phoenix pipeline (https://github.com/tgen/phoenix). Differential expression was performed between Pre and Post groups using DESeq2.

CTC Detection Platform and Workflow

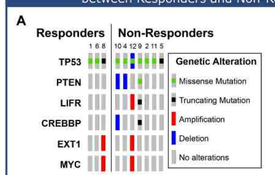
- Blood samples collected Pre-PIKTOR and Post-PIKTOR were analyzed by Epic Sciences for CTC enumeration, cell morphology, phenotypic heterogeneity, and genomic instability (GI) analyses via a previously developed GI prediction algorithm based on cell phenotypes (**Figure 3**).[4]

Table 1. Patient Demographics and Treatment

Patients		Treatment on Study				Post-Study Treatment	
Patient ID	Sites of Metastatic Disease	Total weeks on PIKTOR	Total Weeks on Cis/Nab Pac	Response to Cis/Nab Pac	Progression-Free Survival (weeks)[1]	Treatment	Post-study Treatment Duration (weeks)
001	LN, bone, brain	7	16	SD	28+	Pembrolizumab	103
002	chest wall, LN, lung	9	3	PD	9+	Entrectinib	5
004	LN, lung	10	17	PR	22+	Paclitaxel + Bevacizumab + Pembrolizumab	18
005	LN, lung	5	9	PD	14+	Eribulin + Pembrolizumab	9
006	LN	11	6	PD	19+	Pembrolizumab	69
008	LN	14	18	SD	18+	Pembrolizumab	61
009	lung	4	7	PD	17+	Pembrolizumab	3
010	LN	14	17	SD	17	N/A	N/A
011	LN	7	3	PD	6+	Paclitaxel + Bevacizumab + Capecitabine	2
012	LN, lung	3	6	PD	11+	Erubilin	6

Abbreviations: LN=lymph node; N/A=not applicable; PD=progression of disease; PR=partial response; SD=stable disease
[1]PFS was calculated from C1D1 of cis/nab pac until PD
Highlighted Patients 1, 6, 8 had durable stable disease on cis/nab pac followed by pembrolizumab and are identified as Responders.

Figure 2. Gene Expression and Genomic Alterations in the Pre-PIKTOR Biopsies between Responders and Non-Responders



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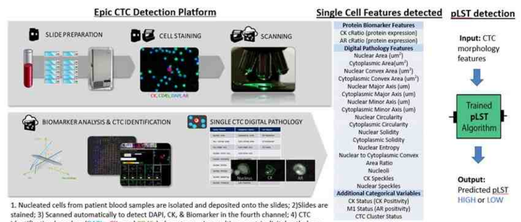
Gene	Log_2 Fold Change	SE	P-value	padj
MYO7B	4.34	1.04	0.0000300	0.0321
TOX	3.27	0.82	0.0000706	0.0464
CLDN18	-25.16	2.66	0.0000000	0.0000
CTSE	-22.65	2.64	0.0000000	0.0000
SFTPA2	-11.84	2.47	0.0000017	0.0036
SFTPA1	-10.06	2.48	0.0000514	0.0428
OGDHL	-9.35	2.18	0.0000173	0.0216
PDE8B	-8.46	2.13	0.0000713	0.0464
GCNT3	-8.36	2.12	0.0000778	0.0485
SEC14L4	-8.23	1.60	0.0000003	0.0010
CXCL17	-8.02	1.62	0.0000007	0.0020
PI3	-7.24	1.63	0.0000091	0.0123
AQP4	-7.15	1.79	0.0000672	0.0464
ALX4	-6.37	1.42	0.0000070	0.0105
C2CD4A	-6.36	1.54	0.0000369	0.0368
GPR143	-6.13	1.24	0.0000008	0.0020
KCNS1	-5.74	1.40	0.0000395	0.0370
VWA2	-5.71	1.42	0.0000597	0.0447
DUSP9	-5.55	1.07	0.0000002	0.0010
ASPHD1	-5.47	1.36	0.0000563	0.0444
PALM3	-5.12	1.12	0.0000046	0.0076
TMEM200C	-4.96	1.05	0.0000022	0.0041
RASL10B	-3.22	0.79	0.0000427	0.0376
BEX3	-2.51	0.60	0.0000276	0.0318

Signal transduction genes
Immune-related genes

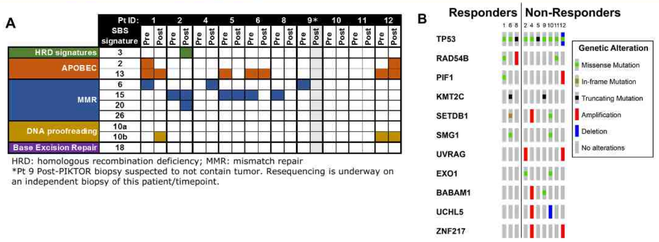
A) Oncoprint of SNVs and CNAs in COSMIC annotated oncogenes and tumor-suppressor genes in pretreatment tumor biopsies, separated by responders and non-responders to cis/nab pac then pembro.
B) Significantly differentially expressed genes between responders vs. non-responders in pretreatment tumor biopsies. Genes more highly expressed in responders are shown in red, and those highly expressed in non-responders are shown in blue.

Figure 3. Epic Sciences CTC Detection Platform and Workflow



1. Nucleated cells from patient blood samples are isolated and deposited onto the slides; 2)Slides are stained; 3) Scanned automatically to detect DAPI, CK, & Biomarker in the fourth channel; 4) CTC identification based on (DAPI+, CK+ and CD45-) phenotype using multiparametric digital pathology algorithm

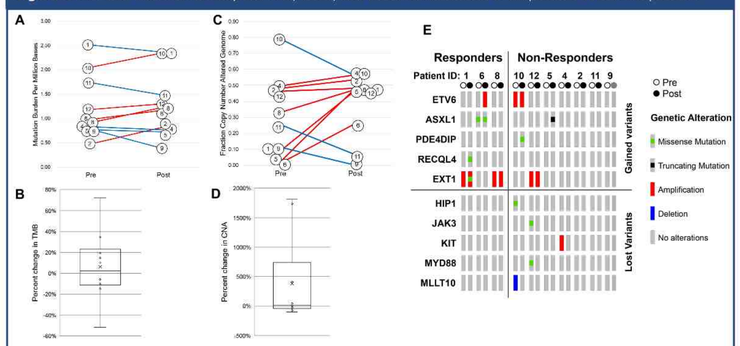
Figure 4. Genomic Alterations Pre- and Post-PIKTOR in DNA Damage Response/Repair Signatures (A) and in DDRD Genes in Pre-PIKTOR Biopsies (B)



HRD: homologous recombination deficiency; MMR: mismatch repair
*Pt 9 Post-PIKTOR biopsy suspected to not contain tumor. Resequencing is underway on an independent biopsy of this patient/timepoint.

A) COSMIC Single Base Substitution (SBS) Signatures (v.3.1) were applied to SNVs identified by WES. Presence of DNA damage repair-related signatures in each patient/sample are indicated by a filled square, absence of signature is white. B) Oncoprint of SNV and CN alterations in DNA damage repair genes (from GO:0006281, 242 genes), plus DNA methyltransferase, SWI/SNF chromatin remodeling complex, and histone deacetylase genes in tumor biopsies prior to PIKTOR treatment. Only genes that were altered in more than one patient are shown.

Figure 5. Pre- and Post-PIKTOR biopsies TMB, CNAs, and Genomic Alterations in Responders vs Non-Responders



A) Pts with increases in tumor mutation burden (TMB) are lined in red; pts with decreases in TMB are lined in blue. B) Box and whisker plot summarizes TMB change post-PIKTOR treatment. Vertical line across the box is the median, "x" is the mean, individual values are "o". Upper and lower quartiles (25 and 75 percentile) are represented by the box top and bottom. C) Pts with increases in fraction of copy number altered (CNA) genome post-PIKTOR are lined in red; pts with decreases in CNAs post-PIKTOR are lined in blue. D) Box and whisker plot summarizes CNA changes post-PIKTOR treatment. E) Oncoprint of somatic mutations and CNAs in COSMIC annotated oncogenes and tumor-suppressor genes that changed pre- and post-PIKTOR treatment.

Table 2. CTC Enumeration, pGI, and Heterogeneity Pre- and Post-PIKTOR

	Pre-PIKTOR				Post-PIKTOR			
Patient ID	Blood Draw Date	CK+ CTC/mL	pGI+ CTC/mL	Phenotypic Heterogeneity	Blood Draw Date	CK+ CTC/mL	pGI+ CTC/mL	Phenotypic Heterogeneity
001	2017-06-19	0.0	0.0	0.00	2017-11-14	4.1	0.0	1.07
002	NA	NA	NA	NA	2017-11-06	3.9	0.0	0.87
004	2017-12-21	0.3	0.0	0.00	2018-02-08	0.0	0.0	0.00
005	2018-02-09	1.0	0.0	0.00	2018-04-13	0.0	0.0	0.00
006	2018-01-19	0.6	0.0	0.00	2018-07-12	0.4	0.0	0.00
008	2018-05-03	13.0	0.0	1.58	2018-08-30	0.6	0.0	0.00
009	2018-06-25	1.8	0.0	1.10	2018-09-12	10.1	0.0	0.00
010	2018-07-20	1.9	2.0	1.04	2018-10-30	2.5	NA	NA
011	2018-07-25	4.0	2.9	1.91	2018-09-21	6.9	3.9	1.01
012	2019-08-27	0.0	0.0	0.00	2019-09-18	0.0	0.0	0.00

NA: blood sample not provided or failed QC
CK+ CTC/mL: CK positive CTC count per mL blood tested
pGI+ CTC/mL: predicted genomic instability positive CTC count per mL blood tested
Phenotypic heterogeneity measured by Shannon diversity index

RESULTS

Clinical Trial

- 10 pts received PIKTOR followed at progression by cis/nab pac (**Table 1**): Median number of prior chemotherapy regimens was 3 (range, 1-5); 7 pts had prior carboplatin; Median time on PIKTOR prior to PD was 8 wks (range, 3-14).
- **With cis/nab pac, 1 pt had PR, 2 had SD > 6 mos, 1 had SD and 6 had PD (Table 1).**
- 2 SD pts (sites LNs +/- bone) and 1 PD pt (sites LNs), all carboplatin-pretreated, whose pre-PIKTOR TNBCs were PDL1-negative (2 pts) or unknown (1 pt) **had durable SD on pembrolizumab post-cis/nab pac for 14+ mos (Pts 1, 6, 8 = "Responders"; Table 1).**
- PIKTOR-related AEs ≥30% included: fatigue (90%); nausea (80%); diarrhea (60%); vomiting (40%); stomatitis (40%); hyperglycemia (30%); rash (30%); cough (30%); chest pain (30%). Incidence and grade of cis/nab pac-related AEs were not greater than expected.

DNASeq and RNAseq

- PTEN inactivation was uniquely observed in the pre-PIKTOR biopsies in non-responders. The immune-related gene TOX, related to T-cell development and regulation of PD-1 expression, was differentially overexpressed in responders' pre-PIKTOR biopsies. Cathepsin E (CTSE), Surfactant A1 and A2 (SFTPA1 and SFTPA2), chemokine CXCL17, and DUSP9, a negative regulator of the MAP Kinase pathway, were differentially overexpressed in the pre-PIKTOR biopsies in non-responders (**Figure 2**).
- APOBEC and MMR, and not HRD, signatures were predominantly expressed in the pre- and post-PIKTOR biopsies. **The 3 responders each lost a defective MMR signature post-PIKTOR (Figure 4).**
- **2 of the 3 responders had a low number of CNAs in pre-PIKTOR biopsies and all 3 demonstrated increased CNAs post-PIKTOR.** Responder Pt 1 had new RECQL4 missense mutation post-PIKTOR, involved in double strand break repair[5] (**Figure 5**).

CTC Analyses

- CTCs pre- and post-PIKTOR in the responders did not show an increase in genomic instability and phenotypic heterogeneity with the exception of Pt 1, who had more CTCs and more heterogeneity in the CTCs in the post-PIKTOR blood sample (**Table 2**).

CONCLUSIONS

- Priming pts' metTNBCs with PIKTOR therapy to increase HRD did not lead to durable responses with cis/nab pac in most pts in this pretreated population.
- 3 of 10 pts (Pts 1, 6, 8) who had carboplatin-pretreated disease in LNs +/- bone, had highly durable SD on pembrolizumab (for 24, 16, and 14 months, respectively) following PIKTOR and cis/nab pac therapy, and were considered to be durable "responders" in this study.
- In the 3 responders, there were substantial increases in copy number alterations in the post-PIKTOR biopsies, suggesting increased "BRCA-ness".
- The 3 responders all had loss of an MMR signature on their post-PIKTOR tumor biopsies compared to their pre-PIKTOR biopsies.

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