

Publication Number: SS1-04

Comprehensive genomic and transcriptomic profiling of molecular subtypes reveal ancestral differences in the activity of signaling pathways between patients with African and European ancestry

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Background: Breast cancer demonstrates heterogeneity in biological features, and the therapeutic strategy depends on tumor subtype. African-Ancestry (AA) patients experience a disproportionately high rate of triple negative breast cancer (TNBC) and worse outcomes than European-Ancestry (EA) patients. However, the biological drivers causing this disparity between ancestral populations are not deeply understood. To address the issue, we performed genomic and transcriptomic sequencing of breast tumors for comparison between AA and EA patients according to breast cancer molecular subtypes. **Materials and Methods:** The study included 221 AA and 341 EA patients. Collected samples underwent the Tempus xT next-generation sequencing panel. Following DNA-panel and whole-transcriptome RNA-sequencing, we compared gene mutation rates, homologous recombination deficiency (HRD) scores, degree of immune infiltration and tumor mutational burden (TMB) between ethnicities and molecular subtypes. Additionally, differences between the activity of relevant signaling pathways were evaluated from RNA-sequencing data. **Results:** Relative to EA TNBC, AA TNBC tumors exhibited higher mutation rates in *TP53* (94% vs 86%), *KMT2C* (17% vs 9%), *APOB* (19% vs 10%), *BRCA2* (11% vs 5%), *EP300* (8% vs 2%), *NOTCH1* (12% vs 4%), and *EGFR* (11% vs 4%). Conversely, AA TNBC tumors had relatively lower rates of *PIK3CA* (10% vs 18%), *RB1* (8% vs 15%), and *NF1* (5% vs 11%) mutations. Among patients with HR+/HER2- breast cancer, AA tumors had higher mutation rates in *CCND1* (23% vs 10%) and *FGF3* (16% vs 10%) than EA tumors, but lower rates in *TP53* (32% vs 39%). HRD scores were higher in TNBC and HR-/HER2+ tumors compared with the other subtypes ($P<0.001$). However, there was no significant difference between the HRD scores of AA and EA tumors within TNBC or HR-/HER2+ populations. The highest percentage of immune infiltration was observed in HR-/HER2+ tumors ($P=0.036$), with no difference between AA or EA groups. TMB did not differ across ancestries or subtypes. Although immune pathways were generally more active in TNBC compared to the other subtypes, there was no difference in pathway-specific immune activation between ethnicities. The G2M and E2F pathways were significantly more active in TNBC ($P<2e-16$ for both), in particular more active in AA than EA tumors (G2M, $P=0.035$; E2F, $P=0.037$). On the other hand, PI3K, ROS, and xenobiotic metabolism (XM) pathways were significantly less active in TNBC compared to the other subtypes (PI3K, $P=2.4e-5$; ROS, $P=0.014$; and XM, $P<2e-16$). Furthermore, these pathways were significantly less active in AA than EA tumors across all subtypes (PI3K, $P=0.026$; ROS, $P=0.00035$; and XM, $P=0.00041$) and within TNBC (PI3K $P=0.012$, ROS $P=0.014$, and XM $P=0.00018$). **Conclusion:** These data demonstrate significant differences in breast tumor heterogeneity and mutation spectrum in TNBC and HR+/HER2- breast cancers between AA and EA patients. Ancestral differences were also observed in the activity of relevant signaling pathways for TNBC. Overall, the results identify previously unexplored pathways and molecular phenotypes of aggressive disease, providing opportunities for development of more effective biomarker informed treatment of breast cancer in diverse populations.

Publication Number: PS5-24

Novel genomic variants and pathways associated with baseline serum thymidine kinase 1 levels in HR-positive HER2-negative MBC patients commencing palbociclib and letrozole

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Background: Cyclin dependent 4/6 kinase inhibitors (CDK4/6i) and endocrine therapy (ET) have improved progression-free survival (PFS) and overall survival in hormone-receptor (HR)-positive metastatic breast cancer (MBC), but progression of disease is inevitable. Serum thymidine kinase-1 (TK1) is a secreted marker of proliferation that is prognostic in patients (pts) with HR-positive, HER2-negative MBC and may be predictive of ET and CDK 4/6i response. PROMISE (NCT0281902) is a prospective study enrolling women with HR-positive MBC starting palbociclib (Pb) + letrozole (L) (1st line) or Pb + fulvestrant (2nd line). We undertook a comprehensive "omic" assessment of blood, tumor, urine and the fecal microbiome in order to identify novel genomic variants and pathways associated with an early decline in TK1 (measured after 2 months) and PFS. Additionally, patient derived xenografts/organoids were generated at baseline and progression to test new therapeutic approaches to overcome resistance to CDK4/6i and ET. We report the initial association between the baseline genomic landscape and baseline TK1 levels. **Methods:** FFPE tumor biopsies were obtained for DNA/RNA sequencing (TempusTM) and blood samples for TK1 (Divitum[®] assay, Biovica) were collected pretreatment (pre-Pb) and after 2 cycles of Pb + ET (post-Pb2). Both whole-exome (exome capture) sequencing (WES) and RNA-Seq used the Integrated DNA Technologies xGen Exome Research Panel v1.0 capture kit. TK1+ disease was defined as > 200 Du/L and TK1- disease as below limit of detection up to 200 Du/L. We tested the association between genomic and transcriptomic characteristics with baseline TK1 data in pretreatment samples where both WES and RNA-seq and TK1 was available. The data were analyzed using bioinformatics pipelines for somatic and germline mutations and copy number alterations. The current analysis focuses on baseline 1st-line pre-Pb omics data in conjunction with baseline TK1 levels. The database was locked for analysis on 5/29/2020. **Results:** Thirty-three pts (median age: 59 yrs.) were evaluable, with paired samples for TK1 in 32. Six pts had TK1+ disease pre-Pb and post-Pb2. Twenty-two pts had TK1- disease pre-Pb and post-Pb2. Four pts had a decrease in TK1 after 2 cycles of treatment that altered the classification from TK1+ to TK1-. Both baseline RNA seq and serum TK1 (n=16) were available for 4 TK1+ and 12 TK1- pts. In this group, 476 genes were differentially regulated (398 upregulated; 78 downregulated). Pathway analysis demonstrated enrichment in complement and coagulation cascade pathway, PPAR signaling pathway, and metabolism-related pathways related to up-regulation of CYP and UGT gene families. Further testing for the association of WES data with baseline TK1+ (n=8) and TK1- (n=16) disease demonstrated somatic copy number variations on chromosomes 6, 11, 12 and 15. *CDK4* copy number gains were observed in 3/8 TK1+ pts and 0/16 TK1- pts. We also observed that somatic mutations (LOH, copy number and/or SNV/INDELs) were more prevalent in the TK1+ compared to the TK1- pre-Pb group in several cancer-associated genes (*FAS* [*p*=0.06] *PTEN*, *PIK3CB*, *NAB2*, *SOX9* and *FAT1* [*p*=0.08], *TP53*, and *MAP2K4* [*p*=0.22]). Conversely, we also noted that 6/7 pts with *GATA3* mutations had TK1- disease (*p*=0.23). **Conclusions:** Using a comprehensive "omics" approach, our data suggest that a secreted biomarker of proliferation (TK1) obtained prior to initiating CDK4/6i and ET for the first line treatment of HR+ MBC is associated with established and novel genes and pathways associated with prognosis of pts receiving ET and CDK 4/6i. Analysis of on-treatment (after 2 cycles) tumor RNA seq and its association with change in TK1 as well as data from the 2nd-line cohort will be presented at the meeting.

Publication Number: PS2-09

Next generation sequencing (NGS) in older adults with breast cancer using tissue-based and circulating tumor DNA (ctDNA) assays

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Background: With advances in next generation sequencing (NGS) and now approved targeted therapy in breast cancer, genomic testing to identify potentially actionable mutations has become a common practice in patients (pts) with advanced breast cancer using both ctDNA and traditional tissue-based assays. Less is known regarding physician practice patterns in obtaining NGS testing and the practical implications of testing in older adults with breast cancer. **Methods:** Pts with advanced breast cancer underwent molecular profiling using a plasma-based ctDNA NGS assay (Guardant360® or Tempus®) between 5/2015 and 5/2020 at Siteman Cancer Center. Pts with advanced breast cancer who underwent genomic profiling using a tissue-based NGS assay (Tempus®) between 12/2017 and 5/2020 at this institution were also included. Clinicopathological histories were obtained from the medical record. Correlations were examined using a Fisher's exact test. **Results:** During 5/15-5/20, 244 pts underwent ctDNA testing and 147 pts had a tissue-based NGS assay performed. There was no significant difference between the number of pts ≥ 65 years-old who underwent ctDNA testing (n=78, 32.0%) and tissue testing (n=37, 25.2%). There was no statistically significant difference between date of metastatic diagnosis and date of NGS testing between the older and younger cohorts. In pts who underwent tissue-based NGS testing, there was no significant difference between site of tissue tested (distant recurrence vs local) in the older and younger cohorts. The most common clinical managements following both ctDNA and tissue-based testing are presented in Table 1. Out of the 391 pts who underwent testing, 27 pts had both ctDNA and tissue-based NGS performed. Pts ≥ 65 were less likely to have both assays performed (n=3, 11.1%; p<0.05). In pts undergoing both assays, there were high concordance rates of *ESR1* (81.5%) and *PIK3CA* (81.5%) mutations. Mean time between tissue and plasma collection for NGS testing in pts undergoing both assays was 356.4 days.

Table 1

clinical management following NGS testing	≥65 years-old	<65 years-old	p value
no actionable mutations	41 (35.7%)	123 (44.6%)	p=0.1
testing results saved for potential future use	27 (23.5%)	45 (16.3%)	p=0.1
change in management	15 (13.0%)	41 (14.9%)	p=0.6

Conclusion: Older adults, who are typically less likely to be included in clinical trials, may still benefit from NGS to reveal potentially targetable mutations. It is reassuring in our cohort that older adults had ctDNA and tissue-based NGS performed at similar rates as part of standard of care treatment. The clinical management following NGS testing was also not significantly different in the older adult cohort. Older adults were less likely to have both tissue and ctDNA testing performed however, given the high concordance rates between tests, this may be less clinically relevant.

Publication Number: PD7-06

Unstable mutational profile and heterogeneity of residual breast tumor following neoadjuvant therapy from comprehensive genomic and transcriptomic sequencing

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Background: In patients with early breast cancer, neoadjuvant therapy is widely performed as standard of care. While the molecular targeting strategy makes progress in HER2 positive breast cancer, the optimal regimens for ER+/HER2- breast cancer (BC) and triple negative breast cancer (TNBC) remain undecided. Furthermore, there are few strategies for patients who do not achieve complete pathological response (pCR) who have worse prognosis. To address these unmet clinical needs, there is urgent need to examine the genomic alterations and immune microenvironment of residual tumors after neoadjuvant therapy. Here, we conducted a comprehensive analysis integrating genomic, transcriptomic, and clinical data to investigate the difference between primary breast cancer and residual disease. **Materials and Methods:** Using the large prospectively ascertained ethnically diverse Chicago Multi-Ethnic (ChiMEC) cohort of 562 participants with integrated genomic data, we identified 176 patients with breast cancer who underwent sequencing with Tempus xT next-generation sequencing panel including DNA- and whole-transcriptome RNA-sequencing. These included 131 primary breast tumors and 45 residual tumors after neoadjuvant therapy. We compared mutation rates between primary tumor and residual tumor in ER+/HER2- BC and TNBC. We also investigated homologous recombination deficiency (HRD) scores, tumor mutational burden (TMB), degree of immune infiltration, and microsatellite instability (MSI), and their association with survival. **Results:** Out of the 176 patients, there were 72 ER+/HER2- BC, 42 TNBC, and 44 HER2 positive cases. Among patients with HR+/HER2- BC, residual tumors had higher mutation rates in *PIK3CA* (61% vs 33%), *CDH1* (33% vs 17%), *CCND1* (39% vs 7%), *FGF3* (28% vs 0%), *FGF4* (33% vs 2%), *FGF19* (33% vs 2%), and *GATA3* (44% vs 7%) than primary tumors, but lower rates in *TP53* (28% vs 48%), *MAP3K1* (6% vs 40%), *KMT2D* (0% vs 33%), *MCL1* (0% vs 30%), *SPEN* (6% vs 20%), *ZFHX3* (0% vs 20%), *ARID1A* (11% vs 16%), *KMT2C* (0% vs 19%), *LZTR1* (0% vs 19%), *BCORL1* (6% vs 17%), *NOTCH3* (6% vs 17%), *FAT1* (0% vs 17%) and *LPR1B* (0% vs 17%). Relative to primary TNBC tumors, residual TNBC tumors exhibited higher mutation rates in *PTEN* (10% vs 3%), *CCNE1* (10% vs 3%), *CIC* (10% vs 0%), and *KMT2D* (10% vs 3%). Conversely, residual TNBC tumors had relatively lower rates of *MCL1* (0% vs 21%), *RB1* (0% vs 12%), *CDH1* (0% vs 12%), *KMT2C* (11% vs 18%), and *PIK3CA* (0% vs 15%), *CDKN1B* (0% vs 12%) and *ETV6* (0% vs 12%). There was a significant trend of higher TMB (>5.0 mutations/megabase, m/MB) associated with improved disease-free survival among the 176 patients ($P=0.031$). TMB was not significantly different between primary tumors and residual tumors, or across subtypes. Microsatellite instability (MSI) status was high in 3 patients (1.6%) and equivocal in 5 patients (2.7%). TMB in MSI-high or MSI-equivocal tumors was significantly higher than TMB in tumors with microsatellite stability (37.7m/MB vs 5.3m/MB, $P<0.001$). HRD scores were highest in TNBC, and lowest in ER+/HER2- breast cancers ($P=0.022$). There was no significant association between the HRD scores and survival. To date, tumors from 106 patients have undergone immune profiling, with estimation of immune cell infiltration, macrophages, B cells, CD4, CD8, and NK cells. Initial analysis showed no association between immune profiling and survival. **Conclusion:** These comprehensive analyses demonstrated that mutation status in HR+/HER2- breast cancers and TNBC differs between primary and residual tumors after neoadjuvant therapy. We identified genomic alterations and pathways in residual tumors that could be further explored as potential targets in the adjuvant setting to improve long term outcomes for patients who do not achieve pCR.

Publication Number: PD9-12

Concordance and use patterns of tissue and cell-free based genomic testing in metastatic breast cancer

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Genomic testing has transformed clinical care in metastatic breast cancer. Cell-free DNA (cfDNA) has made sequential testing feasible, however, concordance between tissue and cfDNA, sensitivity of cfDNA, and optimal sampling frequency have not been examined on large data sets with clinical annotation. Of the 648 genes in the tissue NGS Tempus xT assay, 105 genes are also included in the Tempus xF cfDNA plasma-based test. Both platforms are run on Illumina and detect SNVs, indels, CNVs, and rearrangements/fusions with high sensitivity and specificity (>90%). This study used the Tempus Labs LENS™ tool to identify the most recent ~3,300 metastatic breast cancer patients who underwent clinical testing and had either tissue-based testing, cfDNA testing or both. Of the 212 patients with xF and xT testing, 87 had xT before xF, 135 had xF before xT, and 7 had the testing performed at the same time. The most common pathogenic germline versus somatic alterations on tissue-based testing and their concordance will also be reported. In summary, this data suggests a variable amount of concordance between tissue-based and plasma-based assays. Timing of assay performance and inclusion of germline DNA might play an important role in contributing to these differences which need further exploration.

Table: Count of Reported Variants and xT/xF Concordance by Gene

Somatic Genes	xF frequency (n)	xT frequency(n)	Concordance Between xF and xT
<i>TP53</i>	139	110	43%
<i>PIK3CA</i>	84	80	58%
<i>ESR1</i>	58	41	41%
<i>BRCA2</i>	38	21	7%
<i>ERBB2</i>	37	26	29%
<i>NF1</i>	32	21	13%
<i>RB1</i>	27	24	16%
<i>ATM</i>	30	16	5%
<i>GATA3</i>	21	34	41%
<i>FGFR1</i>	3	35	3%
<i>PTEN</i>	19	30	36%
<i>ARID1A</i>	25	18	23%