Blood tumor mutational burden and blood copy number burden by genome-wide circulating tumor DNA assessment predict outcome and resistance in hormone-receptor positive, HER2 negative metastatic breast cancer patients treated with CDK4/6 inhibitor

Andrew A. Davis[1], Jingqin Luo[2], Tiantian Zheng[3], Lu Tan[3], Amy Wang[3], Rama Suresh[1], Foluso Ademuyiwa[1], Caron Rigden[1], Timothy Rearden[1], Katherine Clifton[1], Katherine Weilbaecher[1], Ashley Frith[1], Pavan K. Tandra[4], Tracy Summa[1], Britney Haas[1], Shana Thomas[1], Leonel Hernandez-Aya[1], Lindsey Peterson[1], Shujun Luo[3], Chao Dai[3], Bonnie L. King[3], Jianjun Yu[3], Pan Du[3], Shidong Jia[3], Jairam Krishnamurthy[4], Cynthia X. Ma[1]

[1]Department of Medicine, Division of Oncology, Washington University School of Medicine in St. Louis, MO; [2]Dvision of Public Health Science, Department of Surgery, Biostatistics Shared Resource, Washington University in St. Louis, MO; [3]Predicine, Inc., Hayward, CA 94545; [4]Division of Oncology/Hematology, University of Nebraska Medical Center, Omaha, NE.

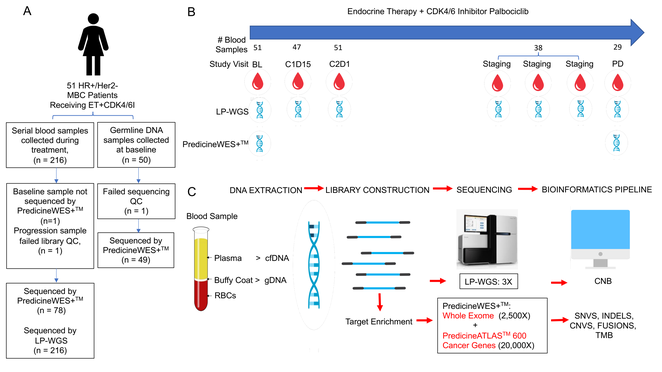
INTRODUCTION

- CDK4/6 inhibitors combined with endocrine therapy improve survival for HR+, HER2- MBC.
- However, biomarkers to predict efficacy and resistance are needed.
- We hypothesized that a comprehensive next-generation sequencing (NGS)-based liquid biopsy assessment of ctDNA mutation and copy number burden may identify novel prognostic and predictive biomarkers.

METHODS

- Serial blood samples were evaluated from the Alt Dose Palbo trial (NCT03007979), a single-arm phase II study of palbociclib plus letrozole or fulvestrant on a weekly schedule of 5 days on/2 days off, in 28-day cycles, as the first- or second-line treatment.
- PredicineWES+™, an assay that combines whole exome sequencing with deep coverage of 600 cancer genes targeted by the PredicineATLAS™ panel, was used to generate genomic profiles of somatic single nucleotide variation (SNV), indels and copy number variation (CNV), and determine blood tumor mutation burden (bTMB) scores reflecting the number of mutations per megabase of DNA.
- LP-WGS was used to generate blood copy number burden (bCNB) scores representing a comprehensive measure of copy number variation, including amplifications and deletions across all chromosome arms.

Figure 1: Study schema and NGS ctDNA analyses.
A) Study schema. B) Sample collection at baseline (BL), and during treatment at cycle 1 day 15 (C1D15), C2D1, Q3-month staging scans without progressive disease (PD), and at imaging detection of PD. C) NGS profiling with PredicineWES+™ and LP-WGS.



RESULTS

Figure 2: High bTMB is associated with poor patient outcomes. A) Distribution of bTMB scores across 50 baseline patient samples sequenced by PredicineWES+™. High bTMB scores were significantly associated with B) lack of clinical benefit (CB) defined as PD within 6 months and C) the presence of *ESR1* mutations at baseline. High bTMB scores were more common in the D) endocrine resistant as compared to the endocrine sensitive cohort (per ESMO 2020 guidelines) and associated with significantly shorter PFS based on the following cutoffs for bTMB: median (E), third quartile (F), and bTMB score of 10 (G).

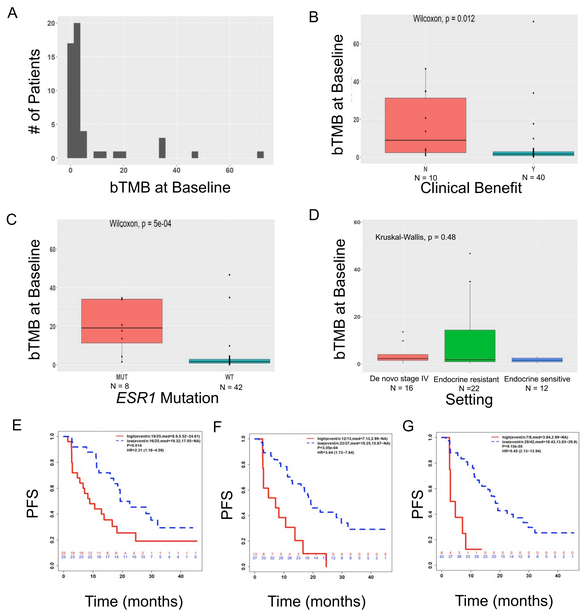


Figure 3: Genomic alterations enriched at progression.
Comparison of the most frequently altered genes detected at progression vs. baseline by PredicineWES+™ across all 28/29 patients who progressed and passed sequencing QC. Enrichment at progression was observed for previously reported alterations implicated in CDK4/6i and ET resistance, as well as for novel alterations in genes outside of the PredicineATLAS™ panel, including *KRT18* and *PABPC3*.

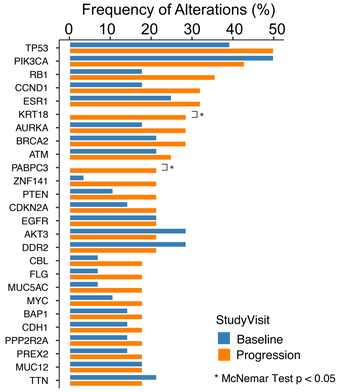


Figure 4: Baseline alterations associated with shorter PFS. Baseline alterations in 91 genes were significantly associated with worse PFS, including alterations previously implicated in CDK4/6i and ET resistance such as *AR*, *ATM*, *AURKA*, *BRCA2*, *CCND1*, *DDR2*, *ESR1*, *FAT1*, *FGFR4*, *FOXP1*, *MYC*, *RB1*, and *RUNX1T1* (A). In addition, baseline alterations in 61 genes outside of the PredicineATLAS™ panel were detected, such as *PLCG1* (phospholipase C, gamma 1) (B).

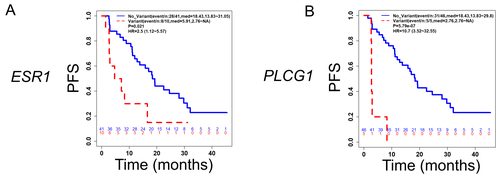
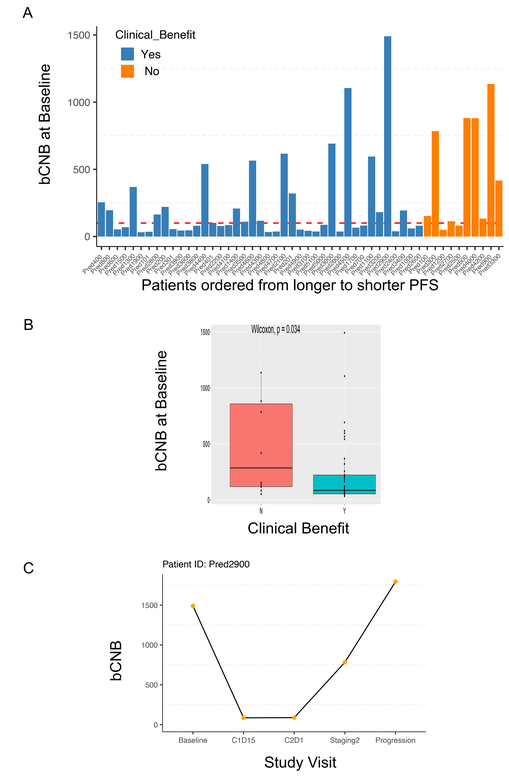


Figure 5: bCNB scores predict clinical benefit and increase before radiographic response and clinical progression. A) bCNB scores across 51 patients at baseline. B) High bCNB scores (>100) were significantly associated with lack of CB. C) Serial analysis of bCNB during treatment revealed decreases at C1D15/C2D1, followed by increases that preceded imaging detection of progressive disease in 9/18 of patients for whom staging blood samples were analyzed.



SUMMARY OF RESULTS

- Patients with clinical benefit had significantly lower bTMB and bCNB scores at baseline.
- Higher bTMB was associated with significantly shorter PFS.
- A majority of patients with high bTMB were endocrine resistant, and high bTMB scores were significantly associated with *ESR1* mutations at baseline.
- Serial bCNB changes demonstrated the potential of a blood-based assay to detect progression prior to imaging.
- Previously reported and novel baseline alterations were significantly associated with shorter PFS.
- Novel alterations were enriched at the time of clinical progression.

CONCLUSIONS

- Using two comprehensive NGS platforms, PredicineWES+™ and LP-WGS, to profile HR+ HER2- MBC patients receiving ET+CDK4/6i we demonstrated that:
  1. High bTMB and bCNB scores identify a subset of patients who do not receive clinical benefit.
  2. Dynamic changes in bCNB scores precede clinical progression.
  3. PredicineWES+™ extends the gold standard for deriving TMB to plasma, detects additional prognostic biomarkers at baseline and reveals novel alterations at progression that may underly resistance.

FUTURE DIRECTIONS

- Determine if HR+ HER2- patients with high bTMB will benefit from novel combination strategies including immunotherapy.
- Further characterize patterns of disease resistance at progression.
- Evaluate bCNB dynamics to predict outcomes and detect progression in an extended cohort.