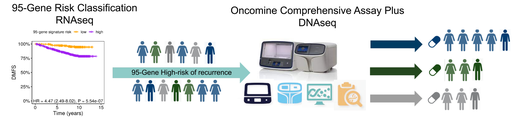
Comprehensive Genomic Profiling of Early Hormone Receptor Positive Breast Cancer Reveals Diverse Relevant Genomic Alterations

John MS Bartlett[1,2], Cheryl Crozier[1], Vinay K Mittal[3], Adam E Sundby[1], Elizabeth Woroszchuk[1], Bradley d'Souza[1], Yu Wang[3], Anna Y Lee[1], Mathieu Larivière[3], Mohit Gupta[3], Sameh El-Difrawy[3], Jeff Smith[3], Santhoshi Bandla[3], Seth Sadis[3], Elizabeth Mallon[4], Annette Hasenburg[5], Christos Markopoulos[6], Luc Dirix[7], Caroline Seynaeve[8], Cornelis J.H. van de Velde[9], Dan Rea[10], Melanie Spears[1,11] and Jane Bayani[1,11]

[1]Diagnostic Development, Division of Adaptive Oncology, Ontario Institute for Cancer Research; [2]Cancer Research UK Edinburgh Centre, Institute of Genetics and Cancer, The University of Edinburgh, Edinburgh, UK; [3]Thermo Fisher Scientific, Carlsbad, CA, USA; [4]Dept of Pathology, Glasgow, United Kingdom; [5]Dept of Gynecology and Obstetrics, University Center Mainz, Germany; [6]National and Kapodistrian University of Athens, Medical School, Athens, Greece; [7]St. Augustinus Hospital, Antwerp, Belgium ; [8]Erasmus MC Cancer Institute, Rotterdam, the Netherlands; [9]Leiden University Medical Center, Leiden, the Netherlands; [10]School of Cancer and Genomic Science, Cancer Research UK Clinical Trials Unit (CRCTU), Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, United Kingdom; [11]Department of Laboratory Medicine and Pathobiology, University of Toronto

Introduction

Breast cancer (BC) has the highest global incidence of all cancer types[1]. Within BC, hormone receptor positive (HR+) BC is the major subtype. Early-stage HR+ BC treated with anti-estrogen therapy has good outcome[1], but depending on initial tumor-node staging, recurrence may occur in 10-40% of cases, representing a major unmet need for precision medicine. In a retrospective analysis, we used comprehensive genomic profiling (CGP) in the well-studied early-stage HR+ BC TEAM cohort to characterize genomic alterations that contribute to oncogenesis and may drive recurrence.



Methods

We used the 95 gene prognostic signature[2] to define low and high-risk early HR+ BC and BC molecular subtypes (Luminal A, Luminal B, HER2 enriched, Basal) from the TEAM trial cohort. The Oncomine™ Comprehensive Assay Plus[3] (OCA Plus) on the Ion Gene Studio™ S5 sequencer[4] was used to characterize genomic alterations. We searched publicly available information to identify driver variants associated with enrollment criteria for open and enrolling BC clinical trials.

Samples

Number of samples sequenced = 1,038 → Number of samples generated sequencing data = 997 (96%) → Number of samples that passed all sequencing QC metrics = 857 (86%)

- Although tumor samples were > 10 yrs old, 96% of the samples generated sequencing data.
- For this retrospective cohort analysis, we used OCAPlus data from 86% of the samples (857 cases) that passed all QC metrics (mapped reads, uniformity, mean read length, MAPD, deamination, etc.).
- 99% of the samples had at least one positive small variant, >50% of the samples had Gene level and Arm level CNV.

Variant type	# samples with positive variants	Variant distribution
Small variants	851 (99%)	Range 1-73, Avg 6.5, Median 5
CNV Amp	425 (50%)	Range 1-27, Avg 5.7, Median 4
CNV Del	264 (31%)	Range 1-33, Avg 2.1, Median 1
ARM Gain	478 (56%)	Range 1-38, Avg 7.9, Median 3
ARM Loss	503 (59%)	Range 1-28, Avg 3.2, Median 2

Small variants, CNV and arm-level aneuploidies contribute to the molecular diversity of early-stage HR positive BC

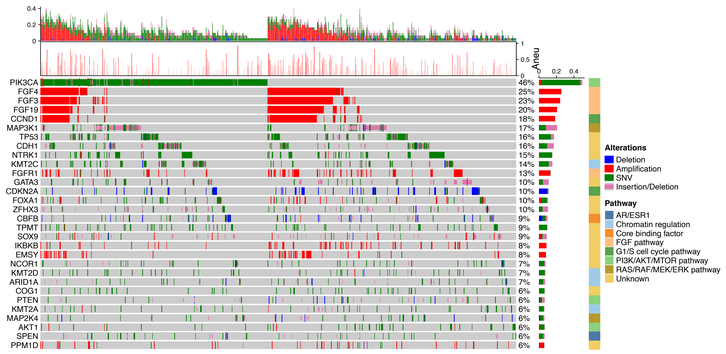


Figure 1. Early-stage HR positive BC OncoPrint[5]. Gene alterations are shown in rows. Aneuploidy score is shown in the top strip. PIK3CA mutation (46%) was the most frequent small variant. Small variants in BC driver genes MAP3K1, TP53, CDH1, GATA3 were prevalent (10-17%). Copy gain of CCND1 and associated FGF3/4/19 and EMSY (11q13), copy gain of FGFR1 and IKBKB (8p11), and copy loss of CDKN2A (9p21) were frequent CNV drivers. Ten samples (1.1%) had high TMB (≥10 mutations/Mb). Several high TMB samples contained mutations in MMR (PMS2 E109GfsTer30), POLE (p.? Splice-site, G1945EfsTer54), and other DNA repair genes.

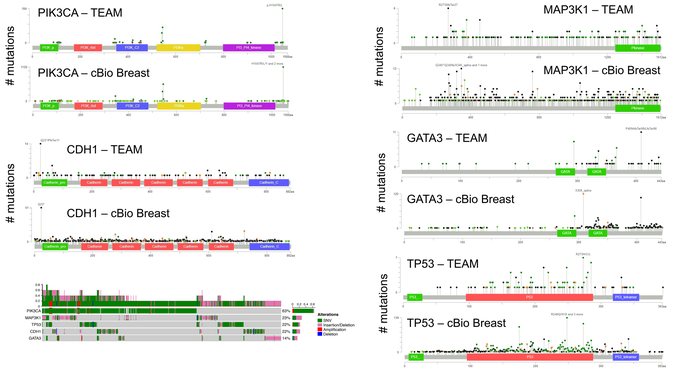


Figure 2. Small variant mutations in BC driver genes PIK3CA, MAP3K1, CDH1, GATA3, TP53 in the TEAM cohort (n = 857) and in the cBio Breast cohort (n = 8881). Whereas PIK3CA was enriched in gain of function hotspot mutations, the BC tumor suppressor genes primarily contained a diverse set of truncating mutations. MAP3K1 and PIK3CA alterations demonstrated co-occurrence (P 0.001) whereas TP53 and CDH1 demonstrated mutual exclusivity (P 0.002). The distribution of small variants detected in the TEAM cohort matches the distribution observed in the cBio breast cancer cohort[6]. (Lollipop plot[7]: missense, truncated, splice, in frame)

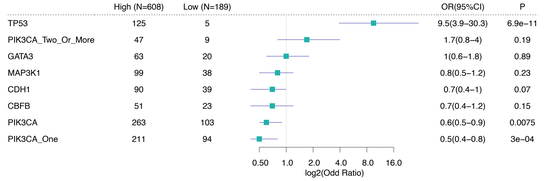


Figure 3. Samples containing small variants in BC driver genes were enriched in BC Risk Subgroups. TP53 alteration was significantly associated with the high-risk subgroup (OR 9.5, P 6.9e-11), whereas PIK3CA alterations and samples with single PIK3CA small variants (OR 0.5, P 0.0075) were associated with low-risk subgroup. Interestingly, samples with 2 or more PIK3CA small variants trended toward high-risk (OR 1.7, P 0.19). Double PIK3CA mutations predict increased sensitivity to PI3Kα inhibitors compared with single-hotspot mutations[8].

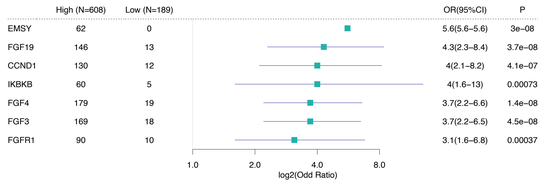


Figure 4. Samples containing copy number gene alterations were enriched in BC Risk Subgroups. Copy gain of CCND1 (OR 4, P 4.1e-07) and associated FGF3/4/19 and EMSY (11q13) and copy gain of FGFR1 (OR 3.1, P 0.00037) and IKBKB (8p11), were associated with the high-risk subgroup.

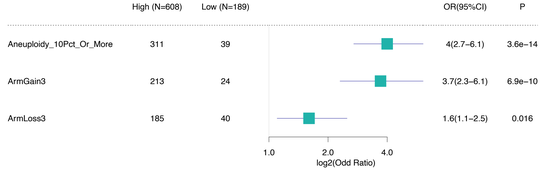


Figure 5. Samples containing arm level copy alterations were enriched in BC Risk Subgroups. Samples with Aneuploidy (≥10% or ≥ 5 arm level alterations) were associated with the high-risk subgroup. Samples containing arm level gain and arm level loss events were mostly distinct. Samples with arm gain events were associated with the high-risk subgroup.

Conclusions

- The genomic landscape of early HR+ BC is diverse and contains many actionable alterations.
- Alterations enriched in the high-risk subgroup included genes with small variants and copy number alterations that are targets for precision medicine.
- The high level of aneuploidy in early-stage HR+ BC was unanticipated and associated with high risk.
- CGP analysis may help address the precision medicine challenge represented by recurrent HR+ BC.

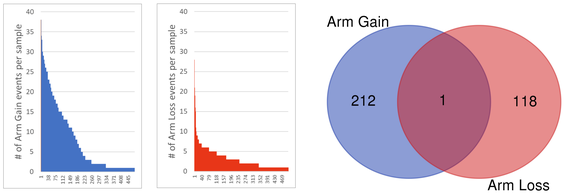


Figure 6. Distribution of arm level alterations in HR positive BC. Left: Although Arm gain and Arm loss events had similar frequency in the cohort (56% and 59%, respectively), the average number of Arm gain events per sample (7.9) was higher than Arm loss events per sample (3.2). Right: High level arm level gain and loss events (≥ 5 per sample) were mostly independent, suggesting different mechanisms.

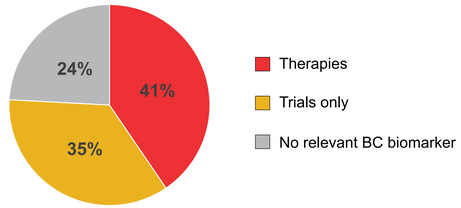


Figure 7. Potential relevance of alterations detected in BC cohort. Alterations were mapped to relevant evidence using publicly available information. Forty-one % of samples contained a biomarker for BC therapies. Thirty-five % of samples had a biomarker that mapped to an open BC trial.

References:
1. Soerjomataram *et al. Breast.* 2022. PMID: 36084384., Ahmed *et al. Current Oncology. 2021.* PMID: 34064867.
2. Bayani *et al. NPJ Breast Cancer. 2017.* PMID: 28649643
3. www.thermofisher.com/us/en/home/clinical/preclinical-companion-diagnostic-development/oncomine-oncology/oncomine-cancer-research-panel-workflow/oncomine-comprehensive-assay-plus.html
4. https://www.thermofisher.com/order/catalog/product/A38194
5. Schlesner *et al. Bioinformatics.* 2016. PMID: 27207943
6. Ellis *et al. Nature. 2012.* PMID: 22722193, Razavi *et al. Cancer Cell. 2018.* PMID: 30205045, Laird *et al. Cancer Cell. 2018.* PMID: 29625048. Cerami et al. *Cancer Discovery. 2012* PMID: 22588877. Gao et al. *Sci. Signal. 2013.* PMID: 23550210. de Bruijn et al. *Cancer Res 2023.* PMID: 37668528.
7. Koeffler *et al. Genome Research.* 2018. PMID: 30341162.
8. Vasan *et al Science 2019.* PMID: 31699932.