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Short Communication

Comprehensive mapping elucidates high risk genotypes in primary metastatic breast cancer

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ABSTRACT

Among women with primary metastatic breast cancer (pMBC), around 5 % of women with primary invasive breast cancer, high-risk mutations associated with disease progression and poor prognosis is shown. The heterogeneity and clinical implications of these genomic alterations remains to be fully elucidated. We performed comprehensive gene mapping on 211 tumors of women diagnosed with pMBC at Rigshospitalet 2014-2021. After DNA purification 203 tumor samples were eligible for analysis. Median age in our cohort was 69 years, 68 % were ER-positive/HER2-negative, 23 % HER2-positive and 9 % triple-negative. A high tumor mutational burden (TMB), shown in 10 %, was in univariable analysis associated with a poor prognosis and a median overall survival of 5.3 months (95 % CI, 2.5-51.3) but no significant association after adjusting for subtype and age. 65 % of tumors had an actionable biomarker, including a *PIK3CA* mutation in 39 %. *TP53* mutations were found in 33 % of tumors and were associated with an increased risk of death (adjusted HR: 1.60, 95 % CI; 1.07-2.40). We have found that for women with pMBC, the disease is driven by several targetable genetic mutations across subtypes, however our results suggest a reduced prognostic value of TMB for this complex patient group. Taken together, our findings substantiate the value of early genomic profiling to actively identify women that may be eligible for a more individualized treatment scheme.

Background

Primary metastatic breast cancer (pMBC) constitutes 5 % of newly diagnosed breast cancer in women, while it accounts for around 30-40 % of all metastatic breast cancers (MBC). pMBC constitutes a particular case of breast cancer as it is treatment naïve as compared to recurrent cases and can thus demonstrate the 'true' nature of metastatic disease. The genomic landscape of MBC has mainly been studied in cohorts including both recurrent and primary metastatic tumors and can be confounded by previous adjuvant or metastatic treatment [1–4]. Using next-generation sequencing panel testing, tumors of primary metastatic and recurrent disease have been compared and demonstrated significant differences in immunohistochemical (IHC) subtypes exhibiting the biological difference between the two types of metastatic disease [5].

In the initial study using comprehensive sequencing, almost 80 % of tumor samples from metastatic triple-negative breast cancers (TNBC) had pathogenic *TP53* mutations. Furthermore, a high frequency of *PIK3CA* mutations (45 %) were found in the ER-positive tumors [1]. These proportions have likewise been seen in pMBC where *TP53* alterations have been associated to shorter survival when adjusting for IHC subtype and *PIK3CA* mutations prevalent in more than 40 % of women with ER-positive tumors [5].

High tumor mutational burden (TMB), defined as ≥10 mutations per megabase, has been correlated to immune checkpoint inhibitor (IO) response and FDA have approved pembrolizumab for high TMB solid tumors [6]. However, the role of TMB remains uncertain in the treatment of women with MBC as high TMB is not routinely tested and treatment with IO is mainly directed by PDL1 positivity [7,8]. However, a recent meta-analysis suggests a limited prognostic relevance of TMB in breast cancer, where better survival is mainly restricted to IO treated

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women with high TMB [9].

In this study we present the results of performing a comprehensive sequencing on a series of unselected, untreated tumors of women diagnosed with pMBC at University Hospital Copenhagen between 2014 and 2021 to further expand the prognostic and treatment related information contained in early genomic profiling for women diagnosed with pMBC.

Methods and materials

Study population

We included identified women aged 18 or above, diagnosed with pMBC at University Hospital Copenhagen, Rigshospitalet between April 1st, 2014, and December 31st, 2021, and for whom sufficient tissue could be obtained from either their primary tumor or from a metastatic site.

Data sources

From the Danish Breast Cancer Group (DBCG) demographic, histopathological and oncological data were retrieved. The following data was obtained: date of diagnosis, age at diagnosis, localization of metastases, ER/HER2 status, date of death. The Danish Civil Registration System by personal identification number was assessed to secure complete follow-up on vital status and emigration until the 1st of June 2024.

Variant analysis

Genomic DNA was extracted from Formalin-Fixed and Paraffin-Embedded (FFPE) tissue samples using the QIACube instrument with the GeneRead DNA FFPE Kit (Qiagen, Hilden, Germany) and quantified using dsDNA High-Sensitivity (HS) or Broad-Range (BR) assay on the Qubit Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA).

DNA libraries were prepared for sequencing from a minimum of 200 ng DNA and hybridized using the TruSight Oncology (TSO) 500 HT gene panel (Illumina) and subsequently, sequenced on the NovaSeq6000 platform to a minimum median coverage of 600 × . For samples with a median coverage below 600 × due to low tumor cell content or poor tissue quality, data was manually inspected for tumor alterations. Sequencing reads were mapped to the hg38/GRCh38 human reference genome using BWA-MEM v0.7.12 software and somatic mutations called using GATK Mutect2 Best Practices guidelines including removal of common polymorphisms present in 1 % of the general population (gnomAD). In addition, the Illumina TSO500 analysis pipeline was applied to estimate the Tumor Mutational Burden (TMB) and gene amplifications (fold change $>$ 2.2 according to manufactures guidelines). TSO500 has been validated in solid tumors for both variants, microsatellite instability (MSI) and TMB [10–12]. All 523 sequenced genes on the TSO500 platform are cancer relevant.

Mutations were identified in QIAGEN Clinical Insight (QCI) Interpret Translational software and manually inspected in Integrated Genome Viewer (IGV) [13]. Pathogenic and likely pathogenic mutations together with rare variants of unknown significance (VUS) were reported. These included nonsense, frameshift, missense, and splice site alterations (+/−2 bp) in cancer-relevant genes with relevant literature and presence of the mutation in cancer tissue (Catalogue of Somatic Mutations in Cancer, COSMIC, https://academic.oup.com/nar/article/47/D1/D941/5146192, accessed on 1 January 2021). High frequency (e.g., more than 10 % of reads) pathogenic and likely pathogenic mutations were included for all reported genes. High frequency VUS were reported with relevant literature (COSMIC) or known breast cancer relevance (e.g., *BRCA1/2* or *PALB2*).

Cut-off values for Mutational Allele Frequencies (MAFs) included MAF $\geq$ 5 %. For mutations with low MAF or coverage or marked by QCI as a possible sequencing artifact or pseudogene was manually inspected in the sequencing reads using the Integrative Genomics Viewer (IGV).

Targetable biomarkers were defined as biomarkers included in OncoKBs Level 1 which includes biomarkers with FDA-approved drugs [14,15].

Statistics

Categorical variables were described by counts and proportions. Differences between high and low TMB in baseline characteristics were tested using Fischer's Exact test or an unpaired t-test (median age). Median overall survival (OS) was reported with confidence intervals and measured using the Kaplan-Meier method [16]. Median follow-up was calculated by the reverse Kaplan-Meier method [17]. Uni- and multivariable Cox proportional hazard regression models were applied to access the relative risk of death (HR). We examined variables deemed relevant for survival after pMBC diagnosis; IHC subtype, age, visceral disease and tumor mutational burden (TMB), and included all but visceral disease in multivariable analysis [18]. The proportional hazards assumption was tested using the Schoenfeld residuals.. In separate models *TP53* and *PIK3CA* respectively mutations were examined both adjusted for age and IHC subtype. All tests were 2-sided, and a P value of $<$ 0.05 was considered statically significant.

Ethical approval

This study was approved by the Ethics Committee of the Capital Region (H-22000267) which waives informed consent of involved women. Furthermore, this study was registered to the Capital Region's research overview (P-2020-861).

Results

From 2014 to 2021, 227 women were diagnosed with pMBC with 211 tumor samples available. After DNA purification, 203 samples (89 %) were eligible and represented the final cohort (Supplementary Figure 1). Median age was 69, most women had ER-positive/HER2-negative tumors and half had visceral disease at diagnosis. High TMB was found in tumors of 21 women (10 %) and these women were both older (median age 78) and with more TNBC than patients with low TMB. Disease presentation did otherwise not differ between the two groups (Supplementary Table 1).

Variant analysis

After curation, 689 variants (pathogenic, likely pathogenic or VUS) were identified within the entire cohort. Two tumor samples did not harbor any variants. Supplementary Table 2 lists the 21 most frequently identified variants and Supplementary Table 3 and 4 display all variants.

In total, 65 % of the analyzed samples had a targetable biomarker (Fig. 1A and B). Almost three quarter of these actionable genes were either a *PIK3CA* mutation or amplification of *ERBB2*, but also *AKT1, PTEN* and *NF1* mutations contributed greatly (Fig. 1C). The heatmap in Fig. 2 correlates mutations, IHC subtype and TMB status. An over-representation of *TP53* mutations were found among the non-luminal subtypes (72 % of TNBC, 61 % of HER2-positives, $p < 0.001$) and most were also TMB high. Among women with ER-positive/HER2-negative tumors several harbor a *PIK3CA* missense mutation (43 %, $p = 0.20$) as are mutations in *CDH1, MAP3K1, GATA3* and *TBX3* mainly found here. Table 1 lists the 10 genes with most variants found (VUS excluded) according to IHC subtype. No microsatellite instability (MSI) was identified in any samples.

Survival

With a potential median follow-up of 70.7 (95 % CI, 62.1-88.7) months the median overall survival was 34.0 (95 % CI, 27.2-48.5)

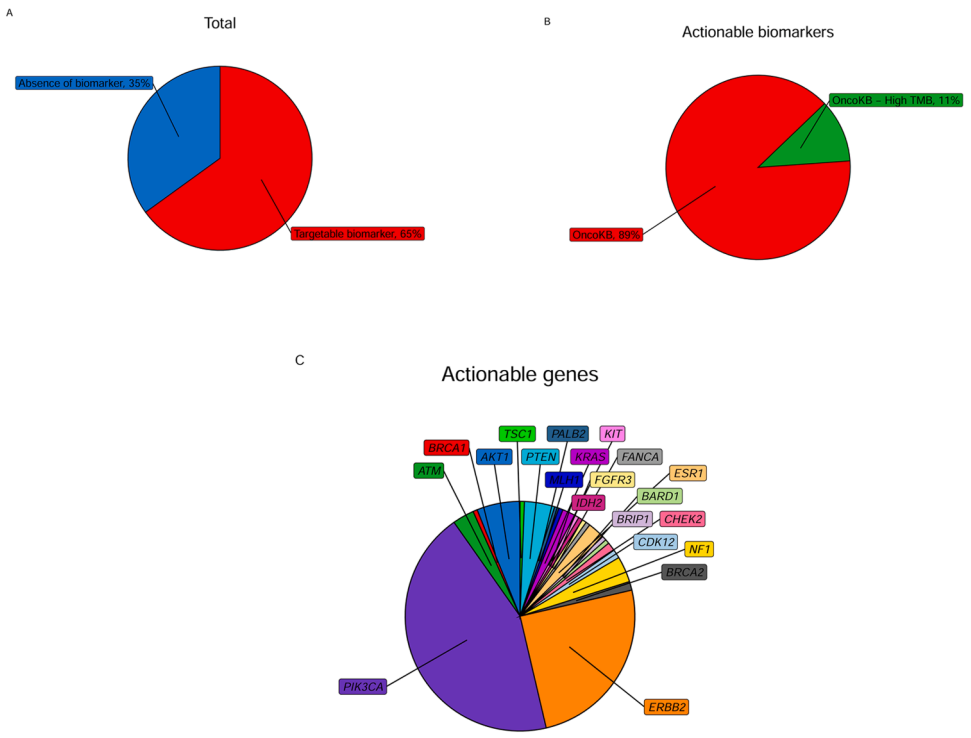

Fig. 1. Distribution of patients with targetable mutation (A), by mutation in OncoKB or High TMB (B) and by specific mutation (C).

months with 147 deaths. Overall survival significantly differed between TMB high and low with a median of 5.3 (95 % CI, 2.5-51.3) and 37.4 (95 % CI, 29.9-50.0) months, respectively ($p < 0.001$) (Supplementary figure 2, Supplementary Table 5). TMB as a continuous variable was significant in a univariable model (HR 1.20, 95 % CI 1.02-1.42), but lost significance when adjusting for age and IHC subtype (HR 1.07, 95 % CI 0.89-1.26). In the multivariable Cox regression model only age and IHC subtype were found as significant factors for survival (Supplementary Table 5).

In a separate model, *TP53* mutation was found to correlate to worse overall survival when adjusting for age and IHC subtype, HR: 1.60 (95 % CI, 1.07-2.40), $p = 0.02$. We did not find any association for *PIK3CA* mutated (HR: 1.08, 95 % CI, 0.77-1.49), $p = 0.69$.

Discussion

We have shown that the 10 % of women with primary metastatic breast cancer and a high TMB have a severely worse prognosis than those with low TMB. Furthermore, 39 % of women harbored a *PIK3CA* mutation, which activate the PI3K/AKT/mTOR pathway, promoting cell proliferation and survival and 33 % a *TP53* mutation a tumor suppressor, leading to uncontrolled cell growth and contributing to cancer progression.

To clarify the extend of targetable mutations with approved treatments in pMBC we compared our mutations to the OncoKB database and found that 65 % of women harbored an actionable mutation mainly driven by *ERBB2* amplification and *PTEN/AKT1/PIK3CA* pathway mutations, but also in other genes with treatments approved for both breast cancer (*ESR1*) and other tumors (*BRCA1/2, KRAS, PALB2*). Additionally, the high frequency of *TP53* mutations in non-luminal subtypes may, depending on whether ongoing drug development succeed in restoring p53 protein function or targeting TP53 mutant cells, become extremely important.

We also investigated the prognostic information contained by TMB and found that when adjusting for IHC subtype and age, no significant prognostic information was contained in TMB (HR, 1.07, 95 % CI 0.89-1.26, $p = 0.50$). To ensure complete coverage of this, TMB was modelled both dichotomous (high vs low) and continuous with no changes in outcome. This lack of prognostic information of TMB was carried by a high representation of TNBC and elderly patients, as these both have an impact on survival. Particularly the risk of death for women with a TNBC in the first year after diagnosis (HR, 3.34, 95 % CI 1.76-6.33) is dominant.

Our study's strengths lie in the unrestricted inclusion criteria which limit the risk of selection bias. Furthermore, we selected samples acquired prior to treatment initiation to avoid any genetic modifications imposed by cytotoxic treatment. With more than 200 samples in an otherwise 'rare' breast cancer occurrence we furthermore provide a rarely seen genomic view in pMBC. Unfortunately, after purification some samples did not contain enough DNA for analysis, and we are limited as some samples were core-needle biopsies which heightens the risk of normal tissue contamination. The sample size, single center setup and lack of external dataset validation may cause an overrepresentation of mutations. The extend of how many women who were treated according to their mutations is limited but may influence survival prediction. Lastly, the TSO500 platform does not support robust detection of deletions and loss of heterozygosity impacting the reporting of tumor suppressor genes.

To our knowledge, previous studies have, as ours, found a high frequency of *PIK3CA* and *TP53* mutations among women with pMBC, often higher than in recurrent or early tumors, a high frequency of targetable mutations and a worse prognosis for women with *TP53* mutated tumors [5,19,20]. The non-significant prognostic information carried in TMB in our results is comparable to previous studies that likewise found a limited prognostic value of TMB [9,21].

Conclusion

Primary metastatic breast cancer harbors several genetic mutations that are accessible, but not readily available, for treatment across all subtypes. Furthermore, this high frequency of actionable mutations promotes the idea of early genetic testing of women with pMBC to

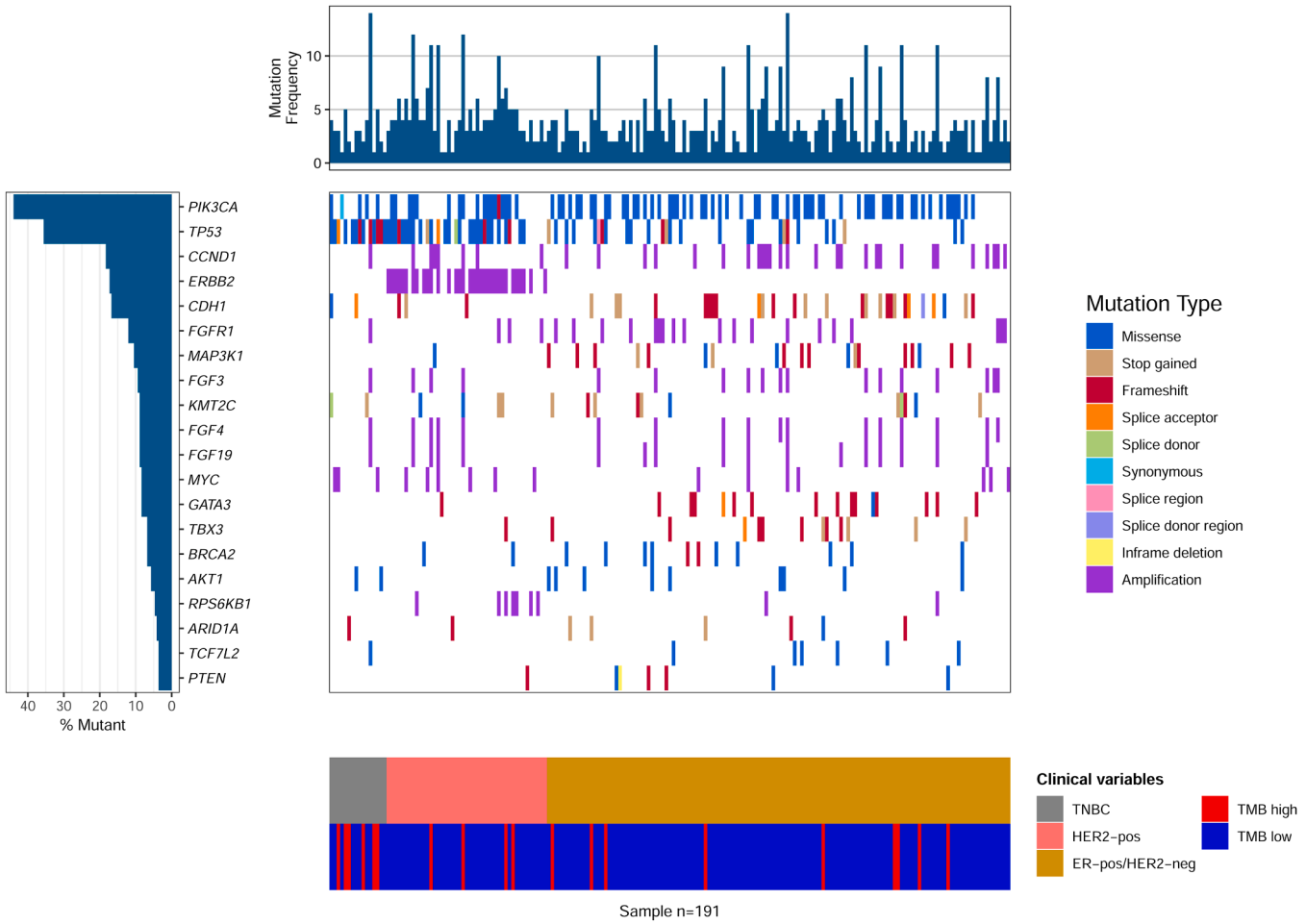

Fig. 2. Oncoplot of the 19 most frequent mutations identified. TNBC: double-negative breast cancer, HER2: human epidermal growth factor rector 2, ER: estrogen receptor, TMB: tumor mutational burden.

Table 1
– Genes with variants (unknown significance excluded) by immunohistochemical subtype.

	TNBC $N = 18$	ER-pos/HER2-neg $N = 139$	HER2-pos $N = 46$	p-value
Gene				
PIK3CA	4 (22 %)	60 (43 %)	16 (35 %)	0.20
TP53	13 (72 %)	26 (19 %)	28 (61 %)	<0.001
CDH1	1 (6 %)	25 (18 %)	3 (7 %)	0.10
MAP3K1	-	16 (12 %)	1 (2 %)	0.06
GATA3	-	14 (10 %)	1 (2 %)	0.13
AKT1	2 (11 %)	9 (6 %)	-	0.08
TBX3	-	11 (8 %)	-	0.07
KMT2C	1 (6 %)	7 (5 %)	2 (4 %)	0.90
ARID1A	1 (6 %)	5 (4 %)	1 (2 %)	0.70
PTEN	-	6 (4 %)	1 (2 %)	0.80

TNBC: triple-negative breast cancer, ER: estrogen receptor, HER2: human epidermal growth factor receptor 2.
d for Eli Lilly and Medac; and has traveled for Daiichi Sankyo and Merck.
MR was on the advisory board for Merck; has conducted talks for Merck and GSK; and has an institutional grant from Astra Zeneca.

identify mutations upfront. Additionally, our results further diminish the prognostic information contained in TMB analysis for breast cancers.

Data availability

Raw reads can be made readily accessible to qualified researchers upon request.

CRediT authorship contribution statement

Tobias Berg: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lise Ahlborn:** Writing – review & editing, Validation, Supervision, Project administration, Data curation. **Maj-Britt Jensen:** Writing – review & editing, Visualization, Methodology, Formal analysis, Conceptualization. **Ann Søegaard Knoop:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Bent Ejlertsen:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization. **Maria Rossing:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

TB has institutional grants from Pfizer, Astra Zeneca, Novartis, Samsung Bioepis, Seattle Genetics, Merck, Eli Lilly, and Daiichi Sankyo. TB was on the advisory board for Novartis and has traveled for Daiichi Sankyo.

LA: None.

MJ was on the advisory board for Novartis.

ASK has institutional grants from Pfizer, AstraZeneca, Merck, Eli

Lilly, Seattle Genetics, Roche, and Novartis and has personal grants from Astra Zeneca (travel and advisory board), MSD (travel), Daiichi Sankyo (advisory board), Novartis (advisory board), Seagen (advisory board), and Gilead (advisory board). BE has institutional grants from: Astra Zeneca, Daiichi Sankyo, Eli Lilly, Merck, Novartis, and Pfizer; was on the advisory board for Eli Lilly and Medac; and has traveled for Daiichi Sankyo and Merck.

MR was on the advisory board for Merck; has conducted talks for Merck and GSK; and has an institutional grant from Astra Zeneca.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neo.2025.101162.

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