High throughput analysis in locally advanced triple negative breast cancer (TNBC): pathological complete response (pCR) and mutational status.

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Background

Pathologic complete response (pCR) after neoadjuvant treatment (NAD) is a strong prognostic biomarker associated with improved survival in breast cancer patients, especially those with TNBC and HER2 positive subtypes. Despite high response rates to NAD, TNBC has been linked to higher rates of recurrence and poorer disease-free survival (DFS) and overall survival (OS). Consequently, there is a continued need to identify prognostic and predictive biomarkers for this population to enhance treatment responses. Next generation sequencing has improved our knowledge regarding the biology of the mechanisms behind treatment resistance. The aim of this study is to evaluate the genomic landscape of tissue samples obtained from locally advanced TNBC patients treated in a NAD setting and to correlate the genomic analysis to response and clinical outcome.

Methods and Materials

We retrospectively evaluated 48 pre-NAD core needle biopsies from a cohort of 136 patients with locally advanced TNBC. pCR and no no pCR was assessed by histology. DNA extracted from archival formalin-fixed paraffin-embedded (FFPE) samples was subjected to NGS sequencing using the TruSight Oncology (TSO) 500 panel (Illumina, San Diego, USA; 523 genes, size: 1.94 Mb), following the manufacturer's protocol, which assesses microsatellite instability (MSI) status, tumor mutation burden (TMB), recurrent somatic copy number variations (sCNV), somatic sequence variants as single nucleotide variants (SNVs) and Insertion/Deletion (InDel). Libraries were sequenced on NovaSeq 6000 Illumina platform to reach a minimum of 500× read depth. We are waiting for RNAbulk analysis. 67 (51.3%) out of 136 patients received a standard anthracyclines/taxane regimen and 69 (50.7%) patients received a platinum-containing chemotherapy regimen. Somatic mutations classified as 'Oncogenic' or 'Likely Oncogenic' according to OncoKB with a variant allele frequency ≥0.03 were included. Differences of mutational profile between pCR and no pCR cohorts were assessed using Chi-squared test. Statistical analysis was performed with R Studio (version 4.2.2).

Results

In total we analyzed samples from 48 patients. The median age was 46 ys. 22 of 48 (46%) were HER2 low, 42 (87%) with G3, 27 (56%) with stage II and 16 (33%) with stage III. 28 out of 48 patients (58.3%) not obtained pCR. Main clinical characteristics of patients and genomic alterations detected are reported in **Table 1**. In the table we show statistically significant correlations between PIK3CA mutations and not pCR. Specifically, the PI3K pathway mutations demonstrate a significant association with not pCR, with a p-value of 0.024. The PIK3CA mutations correlate significantly with not pCR, with p-values of 0.013. The table indicates no statistically significant correlation between BMI, histotype or loco-regional disease extent and the absence of pCR in TNBC patients. These results suggest that PIK3CA mutations and related pathways may impact the effectiveness of NAD in achieving pCR. As shown in **Figure 1** genomic analysis of the entire cohort revealed the most frequently altered genes to be TP53 (94%), PIK3CA (21%), BRCA1 (15%), along with amplifications of MYC (23%) and GATA3 (17%) and MCL1 (15%). Of note, the most aberrant pathways in not pCR population are PI3K and RTK-RAS signaling. Alterations in PIK3CA was observed in 39% of no pCR and absent in pCR patients (p-value < 0.05) as reported in **Table 1**, reflecting its key role in PI3K-AKT-mTOR signaling pathway. The lollipop plots in **Figure 2** show how missense (H1047R, E542K, R88Q, V344G) and inframe deletion (G106_E109del, E110del) mutations are presents in no pCR patients after NAD. Interestingly, median TMB in not responder's cohort is 5.5 while in chemo sensitive patients is 4.8 (p-value 0.93).

	PIK3CA gene	PI3K pathway	RTK-RAS pathway
Age-yrs	p=0,95	p=0,027	p=0,14
Median	48 (25-74)	51 (25-79)	49 (25-79)
BMI	p=0,63	p=0,46	p=0,77
Median	26.5 (18.4-32.0)	26.6 (16.0-35.9)	24.3 (19.1-39.8)
>=30	1 (12%)	6 (27%)	3 (20%)
< 20	2 (25%)	5 (21%)	1 (6%)
20- 30	5 (63%)	12 (52%)	11 (74%)
Smoking status	p=0,68	p=0,21	p=0,44
Smokers	0	0	1 (5%)
Non-smokers	10 (91%)	25 (96%)	19 (95%)
Former smokers	1 (9%)	1 (4%)	0
Stage	p=0,12	p=0,11	p=0,18
I	0	1 (4%)	1 (5%)
II	7 (64%)	15 (58%)	9 (45%)
III	4 (36%)	9 (34%)	9 (45%)
IV	0	1 (4%)	1 (5%)
Grade	p=0,09	p=0,83	p=0,18
G2	3 (27%)	3 (11%)	1 (5%)
G3	8 (73%)	23 (89%)	19 (95%)
Histotype	p=0,73	p=0,71	p=0,58
Ductal carcinoma	9 (82%)	20 (77%)	17 (85%)
Lobular carcinoma	0	0	0
Other	2 (18%)	6 (23%)	3 (15%)
Pathological Response	p=0,013	p=0,024	p=0,048
pCR	1 (9%)	7 (27%)	5 (25%)
nopCR	10 (91%)	19 (73%)	15 (75%)

Tab.1 Clinical characteristics of patients and genomic alterations. Age, BMI, Smoking status, Stage, Grade, Histotype and Pathological response distribution are classified for PIK3CA gene mutations, PI3K and RTK-RAS pathways.

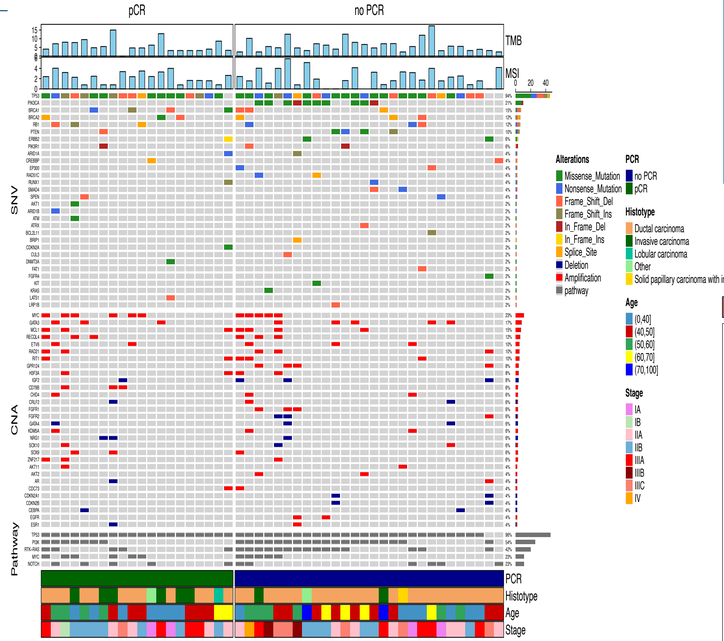


Fig.1 Oncoplot of mutated genes and most altered path in 48 TNBC samples. The genes are ordered from top to bottom by decreasing frequency. ***Top***: TMB and MSI values. ***Bottom***: pCR classification, TNBC histology, Age and Stage color-coded according to the legend. The frequency of mutation in TNBC are shown to the left.

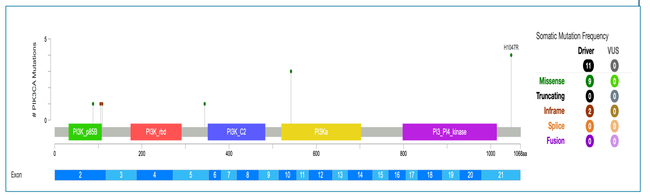


Fig.2 Lollipop plot showing the position of detected missense (9) and inframe deletion (2) mutations in PIK3CA gene sequence.

Conclusions

In this preliminary report, genetic drivers such as PIK3CA may have clinical implications for treatment response following neoadjuvant treatment (NAD) in patients with locally advanced triple-negative breast cancer (TNBC). Aberrant pathways, including the PI3K–AKT–mTOR and RTK–RAS signaling pathways, were predominantly mutated in patients who did not achieve a pathologic complete response (no pCR), suggesting potential avenues for targeted therapeutic interventions. However, a larger sample size is needed to further evaluate the significance of both single mutations and co-alterations in these subpopulation, particularly to identify common co-occurring mutations in different subgroups. We would like to acknowledge the contribution of the Multispecialistic Biobank Research Core Facility G-STeP, Fondazione Policlinico Universitario "A. Gemelli" IRCCS (Biobank-FPG), for providing the bioresources.

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