Biomarker comparison between androgen receptor – positive-triple-negative breast cancer (AR+ TNBC) and quadruple-negative breast cancer (QNBC)

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Abstract

Background: Quadruple-negative breast cancer (QNBC) is a subgroup of triple-negative breast cancer (TNBC) that lacks androgen receptor (AR) expression. While TNBC patients with AR expression have shown a promising response to AR-targeted therapies, QNBC patients' treatment options remain limited, with no targeted therapy. We investigated the biomarker profiles of large cohorts of AR+TNBC and QNBC to identify their molecular differences.

Method: TNBC tumors (defined as negative by IHC for ER, PR, Her2 and ISH for Her2) referred to Caris Life Sciences (Phoenix, AZ) between 2009 and 2015 were evaluated by board-certified pathologists with a combination of immunohistochemistry (AR, cKIT, cMET, EGFR, ER, ERCC1, Her2, MGMT, PD-1, PD-L1, PGP, PR,PTEN, RRM1, SPARC, TLE3, TOPO2A, TOPO1, TS and TUBB3), fluorescent/chromogenic in-situ hybridization, or FISH/CISH, (EGFR, Her2, TOP2A), and sequencing (Next-generation sequencing, or NGS and Sanger). Tumors evaluated included a mix of primary tumors and metastases. QNBC tumors were defined as TNBC tumors that showed negative AR expression (<10% of cells staining).

Results: Among 2,071 TNBC tumors identified, 1,952 tumors had AR IHC performed, out of which 1,612 (83%) were QNBC and 340 (17%) were AR+ TNBC tumors. Tumor expression of PD-L1 (Ab: SP142, Spring Bioscience/130021, R&D Systems, cutoff used: 2+, 5%) was significantly higher in QNBC compared to AR+TNBC tumors (18% vs. 8%, p=0.01), while PD-1 (Ab: NAT105, Ventana) expression on tumor-infiltrating lymphocytes was comparable between the two cohorts (60% vs. 62%). QNBC tumors were significantly more likely to express proteins of cKIT (26% vs. 15%, p=0.01), EGFR (69% vs. 56%, p=0.03), TS (49% vs. 33%, p<0.0001) and TOPO2A (85% vs. 65%, p<0.0001) compared to AR+TNBC. TLE3 expression was significantly higher in AR+TNBC cohorts (48% vs. 32%, p<0.0001). Sequencing reveals that QNBC tumors carried significantly higher mutation rate of TP53 (71% vs. 55%, p<0.0001) while AR+TNBC tumors showed significantly higher mutation rates of PIK3CA (42% vs. 12%, p<0.0001), AKT1 (13% vs. 1%, p<0.0001) as well as ERBB2 (5% vs. 1%, p=0.0003).

Conclusion:
Biomarker comparisons between two molecular subgroups of the TNBC tumors confirm the molecular heterogeneity of this aggressive type of breast cancer. Our biomarker results suggests that for AR+TNBC tumors, future clinical trial design can consider fluoropyrimidines, taxanes, and agents targeting PI3K/AKT/mTOR pathway as well as pan-HER inhibitors, and those agents may be combined with antiandrogen therapies. On the other hand, clinical trials for immune checkpoint inhibitors, TOP2A inhibitors, as well as agents that target cKIT and EGFR should be considered for QNBC tumors. Our findings highlight the molecular differences that should be considered in the design of future clinical trial strategies, warranting further investigation for improving targeted therapy and outcomes in TNBC.

Background

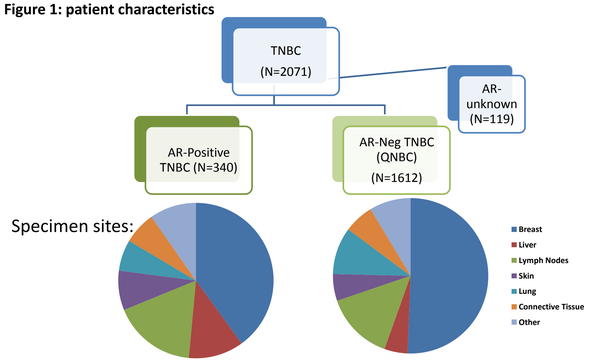
Although TNBC lacks hormone receptors traditionally associated with breast cancer, emerging data suggest that AR plays a strong role in the biology of TNBC and drives a luminal-like expression pattern in the absence of ER (1). Recently reported and ongoing clinical trials using bicalutamide or enzalutamide have shown an increase in PFS, suggesting that AR-targeted therapies may improve patient prognosis and supporting a reclassification of TNBC into AR+TNBC and QNBC (2,3). Potential therapeutic targets have not been systematically explored in QNBC.

Methods

IHCs were performed on formalin-fixed paraffin-embedded (FFPE) tumor samples using commercially available detection kits, automated staining techniques (Benchmark XT, Ventana, and AutostainerLink 48, Dako), and commercially available antibodies. FISH was used for evaluation of the HER-2/neu [HER-2/CEP17 probe], EGFR [EGFR/CEP7 probe], and TOP2A [TOP2A/CEP17 probe] (Abbott Molecular/Vysis). CISH was also used (INFORM HER-2 Dual ISH DNA Probe Cocktail;TOP2A DNP probe, Ventana; EGFR DNP probe, Ventana). NGS was performed using the Illumina MiSeq platform. Specific regions of 47 genes of the genome were amplified using the Illumina TruSeq Amplicon Cancer Hotspot panel. Sanger sequencing included selected regions of BRAF, KRAS, NRAS, c-KIT, EGFR, and PIK3CA genes and was performed by using M13-linked PCR primers designed to amplify targeted sequences.
TNBC was defined as ER IHC negative(Ab: SP1; =0+ or <10%); PR IHC negative (Ab: 1E2; =0+ or <10%), Her2 IHC negative (Ab:4B5) and Her2 ISH negative (Her2 results and interpretation follow the ASCO/CAP scoring criteria. Wolff, AC. et al. (2013) J Clin Oncol: 31 (31):3997-4013). TNBCs with negative AR expression (Ab: AR27; <10% staining) are counted as QNBC.

Results:

Figure 1: patient characteristics



Patient Age	Average	59.2	54.9	p<0.0001
	Interquartile Range	52-68	47-63	

Results

Figure 2: IHC and ISH marker comparison between AR+ TNBC and QNBC. Bars represent the frequency of the biomarker measurement observed in the two cohorts. Arrows indicate that the difference is statistically different (p<0.05) with the corresponding p values labeled. Therapeutic agents listed correspond to agents associated with clinical benefit based on the corresponding biomarkers.

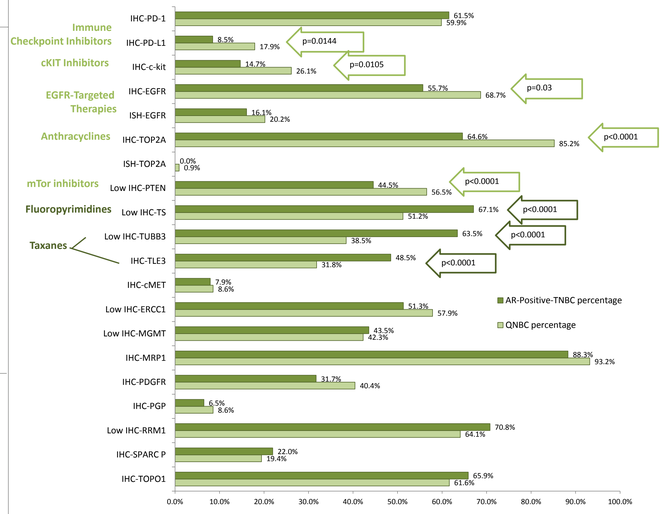


Figure 3: Gene mutations with significant different frequencies between AR+ TNBC and QNBC. Bars represent the frequency of mutations observed in the two cohorts. Arrows indicate that the difference is statistically different (p<0.05) with the corresponding p values labeled.

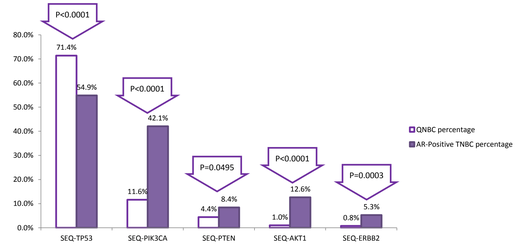
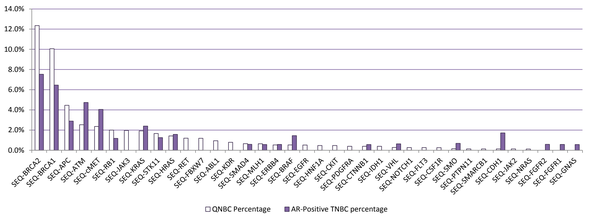


Figure 4: Gene mutations with similar frequencies in AR+TNBC and QNBC. No mutations were observed in ALK, GNA11, GNAQ, MPL or NPM. Bars represent the frequency of biomarker mutations in the two cohorts.



Conclusions

- In a cohort of 1952 TNBC tumors, AR expression was seen in 17.4% of cases, while 83.6% of TNBC were categorized as QNBC tumors.
- Patients with AR+TNBC tumors are significantly younger than patients with QNBC tumors.
- PD-L1 expression is significantly enriched in QNBC tumors compared to AR+TNBC, suggesting activation of this immune suppressive pathway in hormone-independent breast tumors and an increased likelihood of benefit from immune-checkpoint inhibitors in this subtype.
- Overexpression of EGFR, TOPO2A and loss of PTEN expression are more prevalent in QNBC, suggesting additional therapeutic options for QNBC including EGFR-targeted therapies, anthracyclines and PI3K/Akt/mTor inhibitors that should be investigated in clinical trials.
- Low expression of TS, TUBB3 and high expression of TLE3 are more prevalent in AR+TNBC, suggesting potentially increased benefit from fluoropyrimidines and taxane therapies respectively, for this subtype of breast cancer. Combinatorial strategies with these agents and anti-androgen therapies warrant further study in clinical trials.

References

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