Multiplatform molecular profiling of male breast cancer (MBC) reveals significant differences in actionable targets from matched female breast carcinomas

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Abstract

Introduction
Male breast cancer (MBC) is rare, occurring in ~1% of all breast cancers. While clinically characterized as being similar to postmenopausal ER+ BC, MBC has been much less characterized molecularly than female BC.

Methods
60 male (ages 37-84) and 5000 female (ages 27-98) breast cancer samples were evaluated for common gene mutations (Sanger or Illumina Truseq), protein expression (immunohistochemistry), microarray, and/or amplification/rearrangement (CISH or FISH). The samples were analyzed for patterns within the MBC cohort and similarities/differences compared to the female (FBC) subtypes (TNBC, non-TNBC, HER2+, and ER+) evaluated at Caris Life Sciences.

Results
Within the MBC cohort, approximately 10% were negative for ER, PR, and HER2 (TNBC); of those 66% were also negative for AR; 80% were ER+; 51% were both ER+ and PR+. The incidence of high ER and PR protein expression was greater (72% vs. 56%, 54% vs. 40%) but incidence of HER2 overexpression (IHC, 3+) and amplification (FISH, HER2/CEP17 ratio higher than 2) was lower (8.8% vs. 11%, 5% vs. 14.9%) when compared to FBC overall. The rate of EGFR amplification (measured as ≥ 4 copies in 40% or more tumor cells by FISH) was not different from FBC (11%), while the percentage of MBC pts with AR protein expression (74%) was most similar to ER, PR positive FBC patients. Other biomarkers: the rate of ERCC1 overexpression was lower in MBC when compared to FBC (36% vs. 49), the rate of PTEN loss was lower (36% vs. 61%), and the rates of MGMT, TLE3, and RRM1 overexpression was higher (73% vs. 64%, 70% vs. 53%, and 47% vs. 30%, respectively). In the 10 MBC cases evaluated by NGS, no PTEN gene mutations were identified, although PIK3CA gene mutations were seen at a similar rate (50%) as in the >50yo ER+ FBC (37%), and TP53 gene mutations (10%) were seen less frequently than in the >50yo ER+ FBC (27%). Comparison of the TN MBC to the ER+ MBC cohort identified differences in the mTOR pathway (PTEN loss of 17% vs. 28% and PIK3CA mutation rate of 25% vs. 50%, respectively), in P53 mutation rates (33% vs. 0%), and in AR protein expression (33% vs. 82% overexpression), TLE3 (25% vs. 83% overexpression), and ERCC1 (100% vs. 77% low).

Conclusions
The gene mutation, amplification, and protein expression profiles in MBC patients, including HER2 protein expression/amplification, AR and TLE3 protein expression and PIK3CA gene mutation, may inform standard and investigational therapeutic options for this rare cancer population.

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Demographics

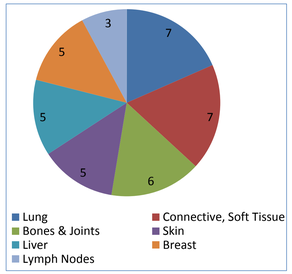


Figure 1. Sites of metastases.

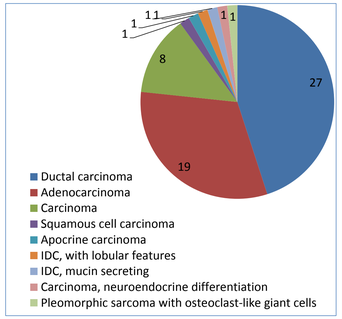


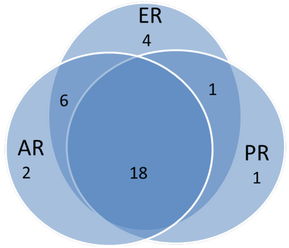
Figure 2. Histologic subtype.

Table 1. Comparison of subtypes found in Caris cohort, compared to FBC and to MBC reported in the literature. MBC most often presents like ER+/HER2-; however, our cohort had fewer ER+/HER2- cases and higher number were triple negative.

Type	FBC	MBC, Caris	MBC, Reported in Literature
ER+/HER2-	60-80%	74%	91-95%
ER-/PR-/HER2-	10-20%	15%	2-4%
ER,PR-/HER2+	10-15%	9%	0-6%
ER+/HER2+	5-10%	2%	0-6%

Results, Hormone Receptor (HR) Status

Figure 3. Co-incidence of AR, ER, and PR. 5 cases were negative for AR, ER, PR, and HER2. Of the 37 cases with all 3 HR's tested, 18 (50%) overexpressed all 3 HR's. We identified overexpression of at least one HR in the 20 other cases where at least one HR was not tested.



Results, Gene Copy Number

Table 2. Changes in gene copy number as measured by FISH or CISH were identified in approximately 20% of cases and were more prevalent in the ER positive, HER2 negative subtype.

Subtype	cMET	cMYC	EGFR	HER2	TOP2A
ER+/HER2-	1/13 (8%)	2/9 (22%)	2/10 (22%)	0%	0%
HER2+	0%	0%	0%	5/5 (100%)	1/2 (50%)
TN	0%	nt	0%	0%	0%

Results, Immunohistochemistry (IHC)

Table 3. Levels of protein expression, reported as percent 'positive' of total cases tested. A. Comparison of MBC to FBC subtypes, and B. Comparison of subtypes within MBC. While MBC has been reported to have more aggressive biology, the overall Ki67 profile was similar in MBC to all FBC profiled at Caris.
$Expression of the biomarker below the threshold is considered predictive of a positive response to therapy.

A. Subtype	n	AR	BCRP	cKIT	cMET	EGFR	ER	ERCC1$	HER2	Ki67	MGMT$	PGP$	PR	PTEN$	RRM1$	TLE3	TOP2A	TOPO1	TS$
MBC	60	74.0%	30.8%	8.0%	18.2%	5.9%	81.1%	37.5%	11.1%	80.0%	70.5%	14.3%	62.4%	62.7%	40.4%	68.2%	58.3%	72.3%	14.6%
All FBC	5500	49%	40%	13%	16%	6%	56%	49%	11%	80%	64%	9%	40%	39%	30%	53%	53%	72%	14%
Triple + FBC	130	79%	26%	2%	21%	0%	100%	63%	63%	66%	69%	4%	100%	52%	27%	70%	57%	75%	11%
non-TN FBC	3525	65%	35%	6%	17%	4%	84%	52%	16%	76%	65%	8%	57%	43%	28%	62%	46%	72%	10%
TN FBC	1975	17%	51%	27%	12%	48%	0%	43%	0%	84%	60%	12%	0%	34%	36%	33%	67%	69%	26%

B. MBC Subtype	# Met (%)	Med. Age	AR	cKIT	cMET	EGFR	ER	ERCC1$	Her2	Ki67	MGMT$	PGP$	PR	PTEN$	TLE3	TOP2A	TOPO1	TUBB3
ER+/HER2- (39)	24 (64%)	65	82.4	0.0	11.8	6.7	100.0	38.9	0.0	81.8	72.4	11.1	74.4	67.6	75.0	66.7	65.6	50.0
HER2+ (5)	4 (80%)	61	75.0	0.0	0.0	nt	80.0	0.0	75.0	100.0	50.0	0.0	60.0	20.0	33.3	50.0	75.0	0.0
TN (9)	6 (67%)	68	44.4	50.0	50.0	nt	0.0	25.0	0.0	50.0	87.5	37.5	11.1	66.7	33.3	20.0	87.5	33.3

Results: Gene Mutations

Table 4. Mutations in a subset of genes were identified. A. Comparison of MBC to FBC subtypes, and B. Comparison of subtypes within MBC. No PTEN mutations were identified in our MBC cohort, and a lower incidence of TP53 mutations was seen compared to previously published. 2 of 3 cases tested had somatic BRCA2 mutations, which is similar to previous findings. A single ERBB2 mutation was found in a triple negative MBC (L869R; previously reported in NSCLC).

A. Subtype	AKT1	APC	CTNNB1	EGFR	ERBB2	HRAS	KRAS	PIK3CA	PTEN	TP53
MBC	0%	0%	10%	0%	10%	0%	0%	50%	0%	10%
All FBC	3%	0.6%	0.0%	0.4%	2%	0.3%	2%	27%	3%	43%
Triple + FBC	0%	0%	0%	0%	6%	0%	0%	38%	6%	50%
non-TN FBC	3%	1%	0%	0%	2%	0%	2%	33%	4%	32%
TN FBC	2%	3%	0.2%	0.4%	2%	1%	1%	13%	4%	64%

B. MBC Subtype	# with Mets (%)	Med. Age	BRCA2	CTNNB1	ERBB2	PIK3CA	PTEN	TP53
ER+/HER2- (7)	3 (43%)	65	66.7 (n=3)	0.0	0.0	53.8 (n=13)	0.0	0.0
HER2+ (0)	2 (67%)	61	nt	nt	nt	66.6 (n=3)	nt	nt
TN (3)	2 (67%)	68	nt	33.3 (n=3)	33.3 (n=3)	25 (n=4)	0.0	33.3 (n=3)

Table 4. Frequency of specific PIK3C*A* mutations in MBC by exon. Mutations were seen in 50% of cases tested; 100% of cases with a PIK3C*A* mutation were also AR+; 6 of 7 were ER/PR+ (86%), and 3 of 9 tested had PTEN loss. In contrast, of the cases with wild type PIK3C*A*, 3 were AR/ER/PR-, 5 were AR/ER/PR+, and only 1 of 10 tested had PTEN loss.

# of Cases	Specific protein change in PIK3C*A* by exon and subtype in MBC
1	N345I, exon 4 (triple-)
4	E545K, exon 9 (triple+)
1	H1047L, exon 20 (HER2-)
4	H1047R, exon 20 (HER2-)

Conclusions

- While few mutations were identified, the multiplatform evaluation of the molecular profiles in MBC identified changes in protein expression which could lead to novel treatment options in MBC.
  - In 98% of cases treatment options were identified based on changes in protein expression or copy number
  - In 17% of cases treatment options were identified based on gene mutations
- The high incidence of AR overexpression warrants continued studies into anti-androgen therapies[3], especially given sequentially with other HR agents, due to the co-occurrence of ER and PR with AR overexpression in 50% of cases.
- HER2 overexpression and/or increased copy number is seen infrequently in MBC; however, when HER2 aberrations are identified, use of HER2 targeted therapies may be efficacious, as seen in recent case reports[4].
- The PI3 kinase pathway (PIK3C*A* mutation or loss of PTEN) was aberrant in 50% of MBC, which may inform use of endocrine therapies in combination with PI3 kinase pathway inhibitors.
- The gene mutation, amplification, and protein expression profiles in MBC patients, including HER2 protein expression/amplification/mutation, ER, PR, AR and TLE3 protein expression and PIK3C*A* gene mutation, may inform standard and investigational therapeutic options for this rare cancer population.

References

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