Genomic and Protein Alterations in 126 Triple Negative (TN) Metaplastic Breast Cancers

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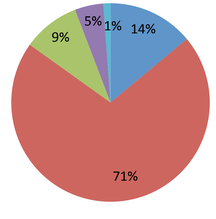
Abstract #1029

Background: Metaplastic breast cancer (MpBC) is a rare subtype (less than 1% of all breast cancers), is generally ER, PR and HER2-negative (TN), demonstrates a claudin-low gene expression profile, and is poorly responsive to cytotoxic therapy. Little is known about the genomic alterations (GA) in MpBC nor about overexpressed proteins that may be amenable to targeted therapy.

Methods: Of 2000 TN breast cancers (TNBC) referred to Caris Life Sciences since 2009 from 50 states and 59 countries, 126 cases were MpBCs based on local pathology evaluation. Specific testing was performed per physician request and included sequencing (Sanger or next generation sequencing [NGS]), protein expression (immunohistochemistry [IHC]), and/or gene amplification (CISH or FISH).

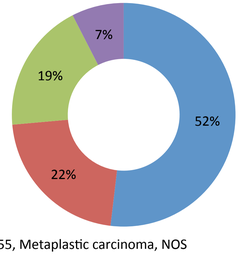
Demographics: Median age=60, range 21-94 (6 patients <50 yo).
81% of patients have documented metastatic disease. Sites of metastasis (if provided) are shown (Fig. 1). The majority of patients had metaplastic carcinoma, NOS or Adenocarcinoma with spindle cell metaplasia (Fig. 2).

Figure 1. Metastatic Sites



n=12, Chest wall, Skin, Soft Tissue
n=61, Not indicated
n=8, Lung
n=4, Lymph Node
n=1, Bone

Figure 2. Patient Distribution by ICD-O code



n=55, Metaplastic carcinoma, NOS
n=23, Adenocarcinoma with spindle cell features
n=20, Adenocarcinoma with squamous features
n=8, Adenocarcinoma with cartilage elements

Results: Table 1 compares the percent gene mutations, amplifications, and IHC findings for biomarkers between TNBC and MpBCs, as a percentage of total patients tested.

TABLE 1.	Gene Mutation, %				ISH, % Positive	IHC, % Positive				
	TP53	PIK3CA	HRAS	cMET	EGFR	PTEN loss	AR	cMET	Ki67	TOPO1
TNBC	64	13	0	0	22	66	17	13	85	70
Metaplastic	32	39	21	4	17	44	8	3	95	49
P value	0.101	0.002	0.002	0.430	0.801	0.001	0.046	0.250	0.650	0.147

- Although not shown, Biomarker profile of MpBC is more similar to non-TNBC than to TNBC
- mTOR pathway involvement (PIK3CA MT and PTEN loss) is significantly different between TNBC and MpBC
- In MpBC cohort, only 2 of 14 cases have PIK3CA and TP53 co-mutated (14%), while in TNBC 26 of 55 cases have PIK3CA and TP53 co-mutated (47%)

Results

Table 2.A. Immunohistochemical analysis of MpBC's. B. Thresholds used to determine biomarker status.

Table 2A	AR	BCRP*	cKit*	cMET	EGFR	ER	ERCC1	HER2	MGMT[$]	MRP1*	p53*
Total Positive	8	7	5	1	7	0	19	0	39	46	20
Total Cases Evaluated	97	11	57	37	9	98	40	99	69	54	42
% Positive	8.2	63.6	8.8	2.7	77.8	0	47.5	0	56.5	85.2	48.6
	PDGFR*	PGP	PR	PTEN[$]	RRM1[$]	SPARC	TLE3	TOP2A	TOPO1	TS[$]	TUBB3[$]
Total Positive	5	8	2	55	20	40	32	37	28	42	17
Total Cases Evaluated	22	82	98	100	63	92	87	58	56	81	25
% Positive	22.7	9.8	2.0	55.0	31.7	43.5	36.8	63.8	50.0	51.9	68

[$]Expression of the biomarker below the threshold is considered predictive of response to therapy;*biomarkers no longer offered.

Table 2B	THRESHOLDS		
AR	=0+ or <10% or ≥1+ and ≥10%	PTEN	=0+ or ≤50% or ≥1+ and >50%
c-kit	=0+ and=100% or ≥2+ and ≥30%	RRM1	=0+ or <50% or <2+ or ≥2+ and ≥50%
cMET	<50% or <2+ or ≥2+ and ≥50%	SPARC	<30% or <2+ or ≥2+ and ≥30%
ER	=0+ or =0% or ≥1+ and ≥1%	TLE3	<30% or <2+ or ≥2+ and ≥30%
Her2	≤1+ or =2+ and ≤10% or ≥3+ and >10%	TOP2A	=0+ or <10% or ≥1+ and ≥10%
MGMT	=0+ or ≤35% or ≥1+ and >35%	TOPO1	=0+ or <30% or <2+ or ≥2+ and ≥30%
PGP	=0+ or <10% or ≥1+ and ≥10%	TS	=0+ or ≤3+ and <10% or ≥1+ and ≥10%
PR	=0+ or =0% or ≥1+ and ≥1%	TUBB3	<30% or <2+ or ≥2+ and ≥30%
ERCC1	<2+ or ≤3+ and <10% or =2+ and <50% or ≥3+ and ≥10% or ≥2+ and ≥50%		

Figure 3. Mutational analysis (Sanger or NGS) of MpBC's.

No mutations were found in the following genes:ABL1,AKT1,ALK,APC,ATM,CDH1,cKIT,CSF1R,CTNNB1,EGFR, ERBB2,ERBB4,FBXW7,FGFR1,FGFR2,FLT3,GNA11,GNAQ,GNAS,HNF1A,IDH1,JAK2,KDR,KRAS,MPL,NOTCH1, NPM1,NRAS,PDGFRA,RET,SMAD4,SMARCB1,SMO,STK11,VHL.

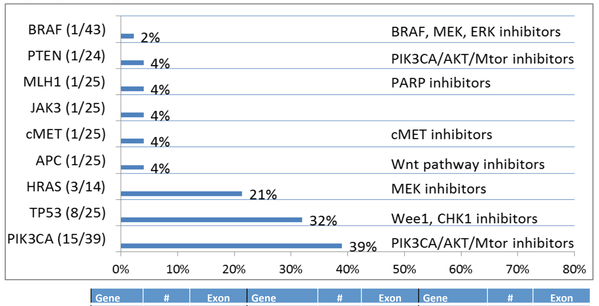


Table 3. Specific mutations in MpBC's identified by gene.

Gene	#	Exon	Gene	#	Exon	Gene	#	Exon
APC			MLH1	#	Exon	JAK3	#	Exon
L1129S	1	16	S406N	1	12	V722I	1	16
BRAF			PIK3CA			TP53		
N581I	1	15	E545K	1	9	S106R	1	4
cMET			G106R	1	1	Y163C	1	5
T1010I	1	14	H1047L	2	20	R213X	1	6
HRAS			H1047R	10	20	G244S	1	7
G12D	1	2	N345K	1	4	Y236S	1	7
G13V	1	2	PTEN			D281E	1	8
Q61L	1	3	R233X	1	7	R273H	1	8
						R333fs	1	10

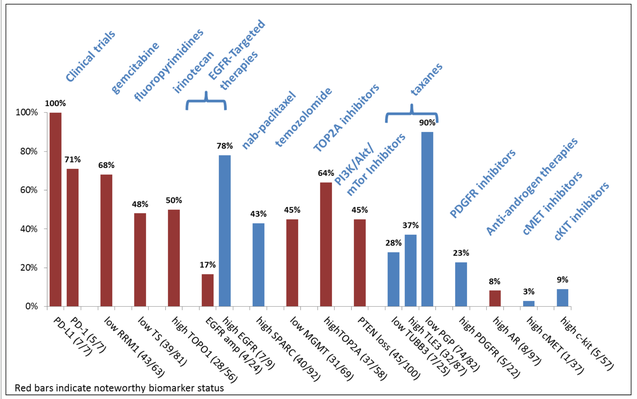
Results

turquoise = change in biomarker relative to therapy or mutation
gray = unchanged or not mutated
light gray = not tested
EGFR/CEP7 ratio ≥ 2 or ≥ 15 *EGFR* copies/cell in ≥ 10% of analyzed cells

Table 4. Comparison of PIK3CA MT vs. TP53 MT vs. EGFR amplified MpBC. Subgroups within MpBC may have different pathways of origin and therapy oppportunities.

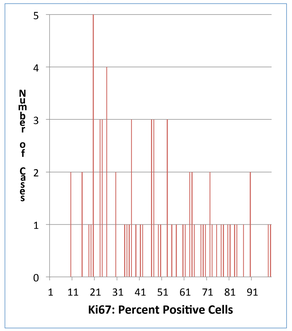
	Demographics				IHC										ISH	DNA Sequencing					
Case #	Histology	AGE	Specimen Site Tested	Metastatic?	AR high	ERCC1 low	Ki67 %	MGMT low	PTEN low	RRM1 low	SPARC high	TLE3 high	TOP2A IHC/ISH	TOPO1 high	TS low	TUBB3 high	EGFR copy #	HRAS	JAK3	MLH1	PIK3CA	PTEN	TP53
PIK3CA Mutated MpBCs, n=14																							
1	Metaplastic, NOS	91	Breast	Y																	E545K		
2	Squamous	71	Skin	Y																	H1047R		
3	Metaplastic, NOS	68	Breast	Y			50														H1047R		
4	Squamous	63	Breast	Y			50														H1047R		
5	Metaplastic, NOS	66	Breast																		H1047R		
6	Metaplastic, NOS	50	Breast	Y																	H1047R		
7	Sarcomatoid	70	Breast																		H1047R		
8	Squamous	57	Breast	Y																	H1047L		
9	Cartilage, osseous	78	Pleura	Y			35														H1047L		
10	Squamous	45	Breast	Y																	H1047R		
																					G106R, H1047R		
11	Squamous	60	Skin	Y																	H1047R		
12	Metaplastic, NOS	65	Breast	Y			58														H1047R		
13	Squamous	50	Breast	Y																	H1047R		
14	Sarcomatoid	86	Breast	Y			20														N345K		
TP53 Mutated MpBCs, n=8																							
15	Metaplastic, NOS	44	Breast																				G244S
10	Squamous	45	Breast	Y																			S106R
11	Squamous	60	Skin	Y																			Y236S
16	Metaplastic, NOS	56	Skin	Y																			D281E
17	Metaplastic, NOS	71	Breast	Y			35																R273H
18	Metaplastic, NOS	68	Skin	Y			81																Y163C
19	Metaplastic, NOS	31	Breast	Y			60																R333fs
20	Metaplastic, NOS	33	Lung	Y																			R213X
EGFR Amplified MpBCs, n=4																							
21	Squamous	65	Breast				34										12.7						
22	Metaplastic, NOS	60	Breast	Y			54										4.19						
23	Sarcomatoid	47	Breast	Y			45										3.91						
24	Sarcomatoid	76	Breast	Y			15										3.66						

Figure 4 - Potential therapeutic strategies suggested by molecular evaluation of MpBC by IHC (immunohistochemistry) and/or ISH (in situ hybridization)



Results

Figure 5. Ki67 analysis. N=64. Median Ki67=46.7. Proliferation of MpBC is highly variable, reflective of the indolent to highly proliferative spectrum of progression seen in MpBC, compared to TNBC, which tends to be more proliferative. 6 cases were both AR positive/ Ki67>20% (median Ki67 for AR+ MpBC=24).



Conclusions

- Although poorly responsive to cytotoxic therapies, molecular alterations were identified in 97% of cases in this large series by multiplatform profiling points to many potential therapeutic strategies for MpBCs, including:
  - mTOR pathway inhibitors: Gene alterations in the PI3K pathway (PTEN/PIK3CA mutations or PTEN loss) (52% of cases)
  - Immunomodulatory agents, currently in clinical trials: presence of PD-1/PD-L1
  - Gemcitabine treatment: Low RRM1 expression in 68% of MpBCs
  - Imitinab or anti-androgen therapies: cKIT (9%) or AR protein overexpression (8%)
  - MEK inhibitors: HRAS (21%) or BRAF mutations (2%)
  - Other potential therapeutically targetable gene alterations are present at low incidence
- Ki67 spectrum reflects variable history and spread between indolent and aggressive progression

References

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3. Hu et al. Current progress in the treatment of metaplastic breast carcinoma. Asian Pac J Cancer Prev. 2013;14(11):6221-5.