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PD2-06

Molecular causes of subtype switching in primary and metastatic breast tumor pairs from the AURORA US Project

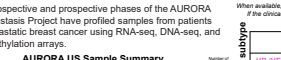
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TBCRC
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Sample Overview

The retrospective and prospective phases of the AURORA US Metastasis Project have profiled samples from patients with metastatic breast cancer using RNA-seq, DNA-seq, and DNA methylation arrays.

AURORA US Sample Summary





In total with RNA-seq data, there are:

- 218 patients
- 161 primary/metastasis (P/M) pairs
- 133 patients with paired P/M samples
- 13 patients with 2+ profiled metastases

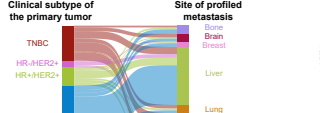
Sample Summary (RNA-seq)

	Primary	Metastasis	Total
Fresh Frozen	11	226	237
FFPE	130	21	151
Total	141	247	388

Anatomic Sites of Metastatic Samples (RNA-seq)

Bone	Brain	Breast	Liver	Lung	Lymph Node	Skin	Other	Total
14	10	9	116	16	29	8	45	247

Subtype Association with Site of Metastasis



Clinical Subtype Switching

When available, the clinical subtype was determined using clinical IHC/FISH data (in parentheses). If the clinical data is not available, we have inferred the clinical subtype from RNA-seq data.

Clinical subtype of the paired metastasis

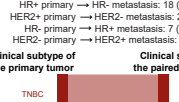
Primary clinical subtype	HR-/HER2-	HR+/HER2-	HR+/HER2+	TNBC	Total	Switches
HR-/HER2+	7 (4)	1		7 (2)	15 (6)	8 (2)
HR+/HER2-	2 (1)	66 (55)	4 (3)	12 (9)	84 (68)	18 (13)
HR+/HER2+	2 (1)	10 (3)	8 (6)	2	22 (10)	14 (4)
TNBC	1 (1)	6 (3)		33 (16)	40 (20)	7 (4)
Total	12 (7)	83 (61)	12 (9)	54 (27)	161 (104)	47 (23)

38/133 (28.6%) patients have clinical subtype switches.
23/104 (22.1%) patients have IHC/FISH clinical subtype switches.

47/161 pairs have clinical subtype switches.
23/104 pairs have IHC/FISH clinical subtype switches.

HR+ primary ⟶ HR- metastasis: 18 (11)
HER2+ primary ⟶ HER2- metastasis: 20 (5)
HR- primary ⟶ HR+ metastasis: 7 (3)
HER2- primary ⟶ HER2+ metastasis: 7 (5)

Clinical subtype of the primary tumor | **Clinical subtype of the paired metastasis**





11 patients (11 pairs) have a HR+ primary and HR- met (IHC).
5 patients (7 pairs) have a HR+ primary and HR- met (inferred status).

Clinical Subtype & PAM50 Subtype Switching

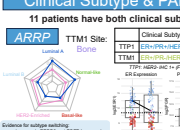
11 patients have both clinical subtype and PAM50 subtype switches.

ARRP

TTM1 Site: Bone

	Clinical Subtype	Inferred Clinical Subtype	Clinical Group	PAM50 Subtype
TTP1	ER+/PR+/HER2-	ER+/PR+/HER2-	HR+/HER2-	Luminal A
TTM1	ER-/PR-/HER2-	ER+/PR+/HER2-	HR+/HER2-	Luminal A-Enriched

TTP1: HER2- IHC 1+ (FISH not performed), TTM1: HER2- IHC 2+ (FISH Amplified) Copy Number = 4.3



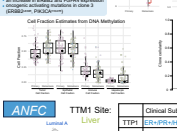
Evidence for subtype switching:
+ an increase in ER882 and FGFR4 expression
+ oncogenic activating mutations in clone 3 (KRAS^{G12D}, PIK3CA^{H1047R})

ANFC

TTM1 Site: Liver

	Clinical Subtype	Inferred Clinical Subtype	Clinical Group	PAM50 Subtype
TTP1	ER+/PR+/HER2-	ER+/PR+/HER2-	HR+/HER2-	Luminal A
TTM1	ER-/PR-/HER2-	ER-/PR-/HER2-	TNBC	Basal-like

TTP1: HER2- IHC 2+ (FISH not amplified), TTM1: HER2- IHC 1+ (FISH not performed)



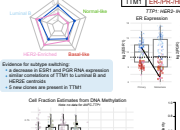
Evidence for subtype switching:
+ a decrease in ESR1 and PGR RNA expression
+ similar correlations of TTP1 to Luminal B and TTM1 to Luminal A
+ 2 new clones are present in TTM1

AR6V

TTM2 Site: Liver

	Clinical Subtype	Inferred Clinical Subtype	Clinical Group	PAM50 Subtype
TTP3	ER-/PR-/HER2-	ER-/PR-/HER2-	TNBC	Basal-like
TTM2	ER+/PR+/HER2-	ER+/PR+/HER2-	HR+/HER2-	Luminal B

TTP1: HER2- IHC 0, TTP2: HER2- IHC 2+ (FISH not amplified), TTM2: HER2- IHC 0



Evidence for subtype switching:
+ a decrease in ESR1 and PGR RNA expression
+ similar correlations of TTP3 to Basal-like, HER2E, and Luminal B centroids
+ high hepatocyte fraction in TTM2

Additional study of the tumors samples from *AR6V* using scRNA-sequencing or spatial analyses will allow us to assess the heterogeneity present in the primary tumor and paired metastasis.

PAM50 Subtype Switching

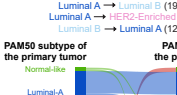
PAM50 subtype of the paired metastasis

Primary PAM50 subtype	Basal	C-low	HER2E	LumA	LumB	Norm	Total	Switches
Basal-like	35		1	1	1	5	43	8
Claudin-low		2					2	
HER2-Enriched			18	1	8		27	9
Luminal A	2		7	26	19		54	28
Luminal B			4	12	15		31	16
Normal-like	2				2	4	4	
Total	39	2	30	40	45	5	161	65

61/133 (45.9%) patients have PAM50 subtype switches.
65/161 (40.4%) pairs have PAM50 subtype switches.

Luminal A primaries are most common in switches (28/65)
Luminal A ⟶ Luminal B (19/28)
Luminal A ⟶ HER2-Enriched (7/28)
Luminal B ⟶ Luminal A (12/16)

PAM50 subtype of the primary tumor | **PAM50 subtype of the paired metastasis**



Conclusions & Future Directions

- Using multi-platform analyses, we have identified clinical and molecular subtype switching in primary/metastasis pairs.
- Clinical subtype switches have implications for clinical treatment as a gain or loss of HR or HER2 expression can change the course of clinical care.
- Subtype switches can be attributed to a combination of (1) changes in tumor biology, (2) normal tissue from the profiled metastatic organ site, and (3) the applied bioinformatic methodologies.
- Our ongoing work and the ARPA-H EVOLVE study (TBCRC) will identify the underlying molecular drivers of subtype switching in patients with metastatic breast cancer and assess treatment strategies.

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