PD2-07

Multi-omics characteristics of ER+/HER2- breast cancer switching to metastatic non-luminal subtype: findings from the AURORA study (BIG14-01)

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Background

- ER+/HER2– breast cancer with luminal primary tumors (P) can undergo intrinsic subtype switching, with metastases (M) displaying non-luminal intrinsic subtypes.
- Non-luminal subtypes are associated with more aggressive behavior and poorer therapeutic responses.
- We investigated the molecular characteristics of intrinsic subtype switching from luminal primary tumors to non-luminal metastases among patients (pts) with ER+/HER2– breast cancer.

Methods

- The AURORA study (NCT02102165) collected multi-omics data from paired primary, metastatic, and plasma samples from 1,148 patients (pts).
- Targeted Gene sequencing (TGS, n=411 cancer genes), Copy Number Alterations by OncoScan (CNA), RNA-seq, and analysis of circulating tumor DNA (ctDNA) by hotspot panel on 21 genes were performed.
- We analysed paired RNA-seq data from pts with ER+/HER2– LumA or LumB (L) primary tumors.
- Intrinsic subtype was assigned based on PAM50 (genefu, robust) and assignments were verified for reliability.
- We defined the following categories:
  - LL: LumA/B in P and LumA/B in M (no switch);
  - LH: LumA/B in P and Her2-enriched in M (switch).
- Only driver mutations, as defined by OncoKB, were considered.
- The lists of pathways and associated genes were manually curated.
- Overall survival (OS) was calculated from the date of diagnosis of metastatic disease.
- Fisher's exact, comet, Wilcoxon, and log-rank tests were used to assess statistical associations where appropriate.

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Results

1. Clinical characteristics

We included 131 pt with ER+/HER2- primary tumors and available RNA-seq in both primary and metastatic samples (**Table 1**).

- 16% (n=21) switched from luminal P subtype to a non-luminal M subtype, mainly HER2-enriched (LH, 14%, n=18), while 84% (n=110) remained luminal (LL).
- Within those with LH switching, IHC subtype switching occurred in 55% of LH, with 33% losing ER and 22% acquiring HER2 (**Figure 1A**).
- Pts with LH tumors had a higher frequency of relapse while on adjuvant endocrine therapy (ET) or within 1 year of ET completion (p<0.001) compared to pts with LL tumors.
- Pts with LH tumors had worse OS than pts with LL tumors, even after excluding pts with *de novo* disease (p<0.001, HR=2.7, 95% CI: 1.5-4.9) (**Figure 1B**).

		LL (n=110)	LH (n=18)
General	Inclusion in AURORA before first line	67 (61%)	14 (78%)
	Inclusion in AURORA after first line	40 (36%)	4 (22%)
	Median age (IQR)	55 (47-65)	60 (53-67)
(Neo)adjuvant Treatment	ET alone	14 (13%)	3 (17%)
	Chemotherapy followed by ET	48 (44%)	14 (78%)
	Neoadjuvant chemotherapy only	2 (2%)	0 (0%)
	ET naive	6 (5%)	0 (0%)
	De novo	40 (36%)	1 (5%)
adjuvant ET exposure	Relapse while on adjuvant ET or within 1 year of completion	39 (35%)	16 (89%)
	Later relapse / ET naive / *De novo*	65 (60%)	1 (5.5%)
	Missing information	6 (5%)	1 (5.5%)

Table 1. Clinical characteristics of patients included in this study. LL: patients with LumA or LumB primary tumors remaining LumA or LumB in metastases. LH: patients with LumA or LumB primary tumors switching to Her2-enriched in metastases.

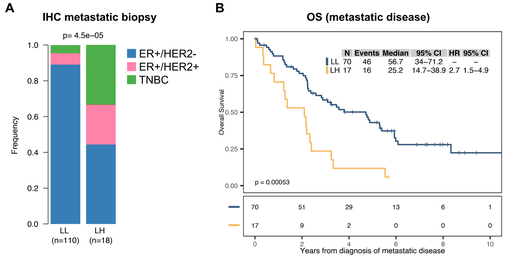


Figure 1. A) Bar plots showing the frequency of IHC subtypes of metastatic samples for non-switching (LL) and switching (LH) tumors. B) Kaplan-Meier curves showing Overall Survival (OS) from diagnosis of metastatic disease for patients with LL and LH tumors. P-value was calculated by log-rank test.

2. Mutational frequency of switchers in P and M

- LH tumors showed a higher frequency of *TP53* mutations in both primary and metastatic samples compared to LL (p<0.001) (**Figure 2A**).
- No significant difference was observed for *PIK3CA* mutations.
- In M, MAPK pathway mutations were more frequent in LH compared to LL tumors (p=0.07).
- No *ESR1* mutations (ctDNA or M) were detected in LH, while 18% of LL were *ESR1* mutated (p=0.02, p=0.01 excluding first line de novo pts) (**Figure 2B**).

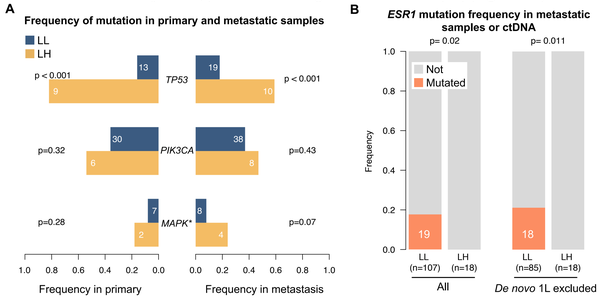


Figure 2. A) Bar plots showing the frequency of mutation of *TP53*, *PIK3CA* and MAPK signaling genes for non-switching (LL) and switching (LH) tumors in primary (left) and metastatic (right) samples. P-value was calculated by Fisher's Exact Test. B) Bar plot showing the frequency of mutation of *ESR1* for LL and LH considering metastatic or circulating tumor DNA (ctDNA) samples, for all patients (left) and excluding first line (1L) *de novo* patients. P-values were estimated by comet test (Leirsen et al. (2015)). *Mutations in *BRAF*, *EGFR*, *ERBB2*, *ERBB3*, *HRAS*, *KRAS*, *MAP2K1*, *NF1* (Razavi et al. (2018)).

3. Transcriptional changes in paired P-M

- Gene expression analysis showed less similarity between P and M pairs in LH compared to LL tumors (**Figure 3A**).
- LH tumors showed decreased *ESR1* expression (paired p<1e-5), increased *ERBB2* (p<1e-4) expression, and increased levels of BC relevant signatures in M compared to P counterparts, with no significant differences in P between LL and LH tumors (**Figure 3B**).

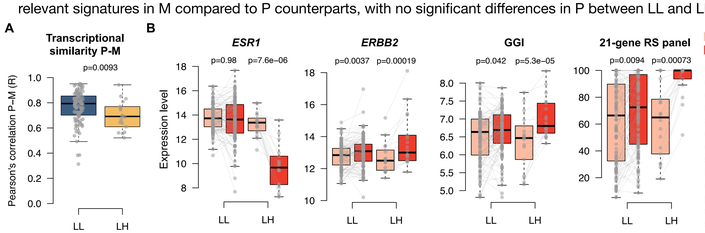


Figure 3. A) Box plots showing the distibution of the Pearson's correlation coefficient R between primary and metastatic samples in non-switching (LL) and switching (LH) tumors. P-value was calculated by Wilcoxon test. B) Gene expression levels of *ESR1*, *ERBB2* and signature level of GGI and 21-gene Recurrence Score (RS) panel (surrogate of OncotypeDx, from genefu) in paired primary (light red) and metastatic (red) samples for non-switching (LL) and switching (LH) tumors. P-values were calculated by Wilcoxon test.

4. Pre-existent mutational determinants of LH tumors

LH primary tumors displayed more frequent alterations in PI3K/AKT and p53 pathways in primary samples compared to LL that acquired *ESR1* mutation in metastasis (**Figure 4**).

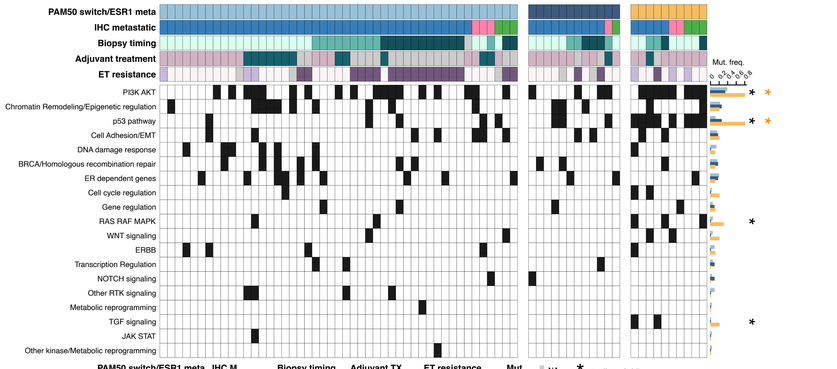
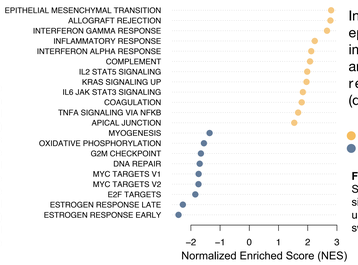


Figure 4. Oncoprint showing alteration frequency of a list of curated pathways in primary samples for non-switching tumors with no *ESR1* acquired mutation in metastatic samples (LL/wt), non-switching tumors with acquired *ESR1* mutation in metastatic tumors (LL/mut) and switching tumors (LH). P-value was calculated by Fisher's Exact Test considering the three categories (black) or LH versus LL/mut (yellow). Only patients with paired P–M TGS samples available were included. PI3K AKT pathway includes: *FOXO1, FOXO3, IRS2, PIK3C2B, PIK3R2, SGK1, AKT1, AKT2, AKT3, INPP4B, PIK3CA, PIK3CB, PIK3CD, PIK3CG, PIK3R1, PTEN;* p53 pathway includes: *PML, MDM2, MDM4, TP53.*

5. Pre-existent gene signatures associating with LH



In P, LH had higher expression levels of epithelial-mesenchymal transition and immune (q<1e-15) expression signatures and lower of levels of estrogen receptor response expression signatures (q<1e-10) compared to LL (**Figure 5**).

- Hallmarks over-expressed in LH vs LL
- Hallmarks over-expressed in LL vs LH

Figure 5. Dot chart showing Normalized Enriched Score (NES) for hallmarks gene-expression signatures significanty over-expressed (yellow) and under-expressed (blue) in switching (LH) versus non-switching (LL) tumors.

Conclusion

ER+/HER2- tumors that switched from luminal P to a HER2-enriched M subtype had worse clinical outcomes and distinct molecular profiles, including *TP53*/MAPK mutations and immune related signatures already present in primary tumors, and reduced ER signaling and higher *ERBB2* expression in metastases. *ESR1* mutations were found only in tumors that remained luminal, suggesting that the luminal to HER2-enriched subtype switch may represent a distinct mechanism of ET resistance. Our findings highlight key features of primary tumors that may predict this subtype switching, guiding future research toward tailored (neo)adjuvant strategies.