Comparative analysis of protein and transcriptomic expression of biomarkers and therapeutic targets in patients with metastatic hormone receptor-positive breast cancer of no-special type versus invasive lobular breast cancer

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INTRODUCTION & OBJECTIVES

- The two major histological subtypes of breast cancer (BC) are
  - Invasive Breast Cancer of No Special Type (IBC-NST), also referred to as "invasive ductal carcinoma"; ~80%
  - Invasive Lobular breast Cancer (ILC); ~15%[1]

- They can either present as a pure form in the primary tumor or, in 5% of all primary BC diagnoses, as a mixture. Mixed IBC-NST/ILC is defined as a tumor with at least 10% of both components present. A previous study of ours showed that the metastases of this mixed subtype displayed mostly a lobular histology.[2]

- IBC-NST and ILC are most often hormone receptor-positive and HER2-negative, however these two subtypes have distinct characteristics. Compared to IBC-NST, ILC is characterized by:
  - Older age at diagnosis
  - Higher stage at diagnosis with more lymph node positivity
  - Less immune infiltration[3]
  - Diffuse metastatic pattern with i.e. more often metastases in bone, brain, digestive tract and reproductive organs[1,2,4]

- Although ILC is the second most common histological subtype of BC, there is a lack of dedicated research to metastatic ILC and it is up until today treated similarly to metastatic IBC-NST.[5,6]

- Research in metastatic BC is hampered by e.g. the lack of samples and to overcome this hurdle rapid autopsy programs or post-mortem tissue donation programs are set up. Since November 2020, our post-mortem tissue donation program, UPTIDER (NCT04531696) has allowed us to do extensive sampling of metastatic BC.[7]

Objective: In metastatic IBC-NST and metastatic ILC, we compared the expression levels of
- clinically relevant biomarkers (sTIL, ER, PR, HER2) on the protein level
- antibody-drug conjugates (ADC) targets and immune checkpoint markers (ICM) on the transcriptomic level

PATIENTS & METHODS

We included all metastases from 25 UPTIDER patients diagnosed with primary hormone receptor-positive BC and categorized them for our analyses according to the histological subtype of the post-mortem metastases:

16 patients with primary IBC-NST

PatientID	HR status	Age at diagnosis
Pt2002	ER+/PR+	39 years
Pt2007	ER+/PR+	68 years
Pt2008	ER+/PR+	69 years
Pt2009	ER+/PR+	51 years
Pt2010	ER+/PR+	65 years
Pt2014	ER+/PR+	48 years
Pt2016	ER+/PR+	49 years
Pt2020	ER+/PR+	64 years
Pt2026	ER+/PR+	47 years
Pt2027	ER+/PR+	65 years
Pt2029	ER+/PR+	35 years
Pt2030	ER+/PR+	41 years
Pt2034	ER+/PR+	44 years
Pt2035	ER+/PR+	48 years
Pt2037	ER+/PR+	43 years
Pt2041	ER+/PR+	42 years

Median: 48 years

7 patients with primary ILC

PatientID	HR status	Age at diagnosis
Pt2005	ER+/PR-	53 years
Pt2011	ER+/PR+	51 years
Pt2012	ER+/PR+	37 years
Pt2023	ER+/PR-	70 years
Pt2025	ER+/PR+	44 years
Pt2039	ER-/PR-	83 years
Pt2040	ER+/PR+	42 years
Pt2044	ER+/PR+	47 years

Median: 49 years

2 patients with primary mixed IBC-NST/ILC*

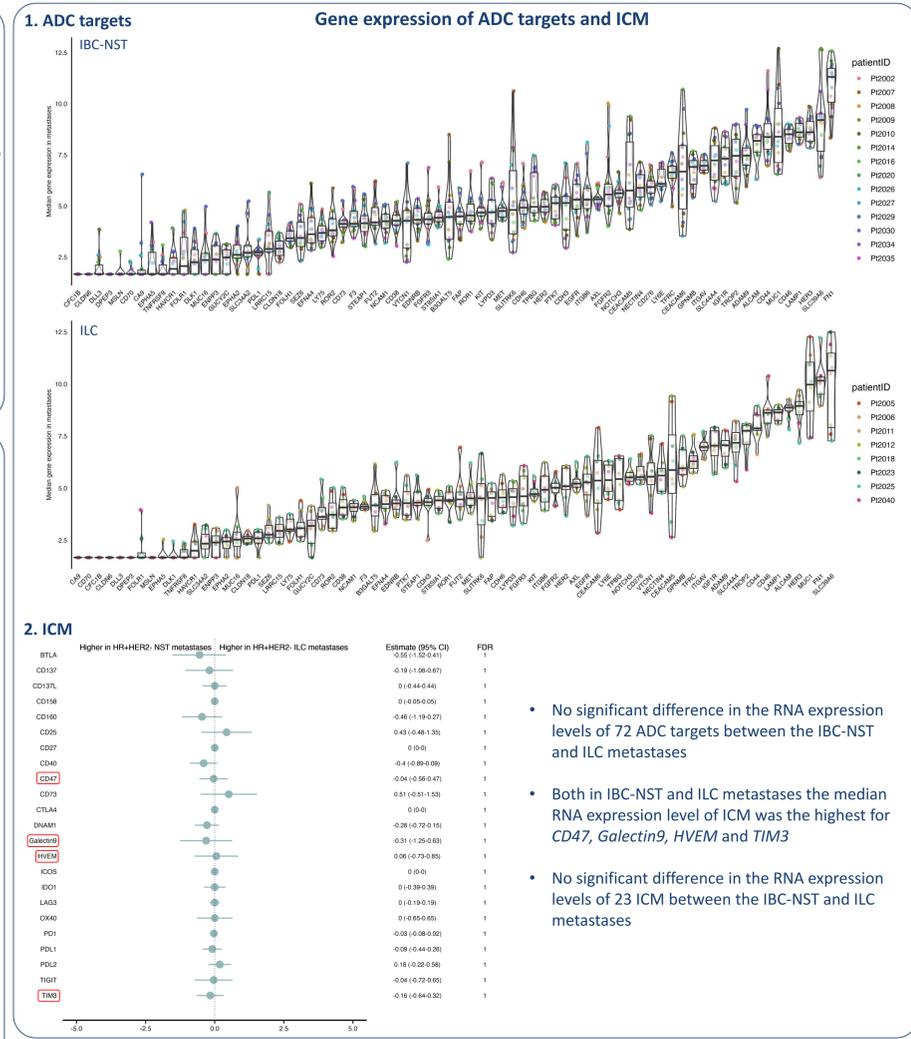| PatientID	HR status	Age at diagnosis
Pt2006	ER+/PR+	46 years
Pt2018	ER+/PR+	47 years

*had either IBC-NST or ILC metastases

Biomarkers		Scoring method	Antibody
H&E	sTIL	Guidelines international sTIL / working group[8]	
IHC markers	ER	ASCO/CAP guidelines 2020[9]	clone EP1, DAKO, RTU, CE-IVD
	PR	ASCO/CAP guidelines 2020[9]	PgR 1294, DAKO, RTU, CE-IVD
	HER2	ASCO/CAP guidelines 2023[10]	anti-human c-erbB-2 protein, DAKO, 1:200, CE-IVD

IBC-NST metastases = 444
ILC metastases = 294

72 ADC targets identified on Clinicaltrials.gov (March 4th 2024 for ADC targets and February 2023 for ICM markers) and currently under clinical investigation in solid tumors

23 ICM targets

FFPE = formalin-fixed paraffin embedded
H&E = hematoxylin-eosin
sTIL = stromal tumor-infiltrating lymphocytes
IHC = immunohistochemistry
ER = estrogen receptor PR = progesterone receptor
HER2 = human epidermal growth factor receptor 2
OCT = optimal cutting compound
ADC = antibody-drug conjugates
ICM = immune checkpoint markers

Statistical analyses:
- Violin plots and bar plots: created with ggplot2 (v.3.4.3)[11] package using standard graphics filters in R (v.4.3)[12]
- Linear mixed quantile regression analysis to observe relevant association between dependent variables (% of cells positively stained (ER, PR, HER2) or mean proportion (sTIL, gene expression), independent co-variates (IBC-NST vs ILC) and random effect on patient ID
- For the expression of ICM: p-values derived from Wald's test and a false discovery rate (FDR) of <0.1 was applied.

RESULTS

Organ involvement at autopsy

- Our cohort showed extensive metastatic disease.
- Liver more often involved in metastatic IBC-NST (n=14/16) compared to metastatic ILC (n=4/9).
- Metastatic ILC more often involvement of the digestive tract (e.g. stomach n=6/9) and reproductive organs (e.g. uterus n=2/9) compared to metastatic IBC-NST (respectively, n=1/16 and n=0/16)

Clinically relevant biomarkers

1. sTIL (n = 325, n = 223)

- The median level of sTIL in:
  - IBC-NST metastases was 1,8% (range: 0-25%)
  - ILC metastases was 0,3% (range: 0-13,3%)
- Significantly lower level of sTIL in ILC metastases compared to IBC-NST metastases (Estimate -1,18; range -1,67 - -0,68; p<0,001)

2. ER (n = 296, n = 242)

- At autopsy, ER-negative metastases:
  - IBC-NST: median of 11,3% (range 4,8-84%) in 4/14 patients
  - ILC: median of 32,3% (range 5,6-61,5%) in 5/9 patients
- The median percentage of ER-expressing cells in:
  - All IBC-NST metastases was 70% (range: 0-100%)
  - All ILC metastases was 50% (range: 0-100%)
- No statistical evidence for lower % of ER-expressing cells in ILC metastases compared to IBC-NST metastases

3. PR (n = 183, n = 210)

- At autopsy, PR-negative metastases:
  - IBC-NST: median of 79,3% (range 12,5-100%) in 10/11 patients
  - ILC: median of 75,7% (range 4,2-100%) in 6/7 patients
- The median % of PR-expressing cells in:
  - All IBC-NST metastases was 0% (range: 0-90%)
  - All ILC metastases was 0,75% (range: 0-100%)
- No significant difference in the % of PR-expressing cells between IBC-NST metastases and ILC metastases

4. HER2

At autopsy,
- HER2-low metastases:
  - IBC-NST: median of 12,1% per patient (range 0-70,5%)
  - ILC: median of 14% per patient (range 8,3-26,7%)
- HER2-ultralow metastases:
  - IBC-NST: median of 17,8% per patient (range 0-50%)
  - ILC: median of 21,7% per patient (range 13,3-65%)

Gene expression of ADC targets and ICM

1. ADC targets

- No significant difference in the RNA expression levels of 72 ADC targets between the IBC-NST and ILC metastases

2. ICM

- Both in IBC-NST and ILC metastases the median RNA expression level of ICM was the highest for CD47, Galectin9, HVEM and TIM3
- No significant difference in the RNA expression levels of 23 ICM between the IBC-NST and ILC metastases

CONCLUSIONS

- This work reports on the differences in the expression of clinically relevant biomarkers and therapeutic targets of interest between patients with IBC-NST and ILC metastases in a post-mortem cohort.

- Metastases in organs less often involved metastatic BC (e.g. stomach) are confirmed in patients with metastatic ILC.

- As described in primary[3], ILC metastases show less immune infiltration (sTIL) compared to IBC-NST metastases. Analysis of the phenotype of the present immune cells still needs to be done.

- Hormone receptor expression "change" from positive to negative is present in both subtypes in the metastatic setting. There was no significant difference in the receptor expression between IBC-NST and ILC metastases.

- All patients with ILC had at least one HER2-low metastasis and HER2-ultralow metastasis, which is relevant given the recent approval of anti-HER2 ADC in these immunohistochemical scoring categories.

- Amongst the ADC targets and ICM, there were no significant expression differences between both subtypes, however, for the ADC targets, FN1 and SCL39A6 and, for the ICM markers, CD47 and Galectin9 appear to be interesting targets for metastatic BC in general and further research is warranted.

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