Metastatic breast cancer is associated with increased levels of soluble inhibitory immune checkpoint proteins and decreased levels of soluble stimulatory immune checkpoint proteins.

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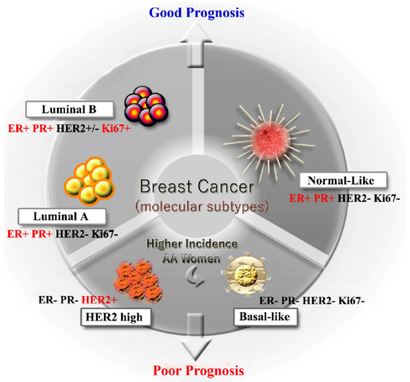
Background

- Female breast cancer (BC) has surpassed lung cancer as the most commonly diagnosed cancer, with an estimated 2.3 million new cases (11.7%) deaths 684,996 (6,9%) [Global Cancer Statistics 2020].
- Heterogeneity of breast cancer – individualized treatment approaches but also challenges.
- Molecular subtypes not only determine the clinical characteristics, treatment and prognosis of the patient, but also the tumor-infiltrating lymphocytes found in the tumor microenvironment.

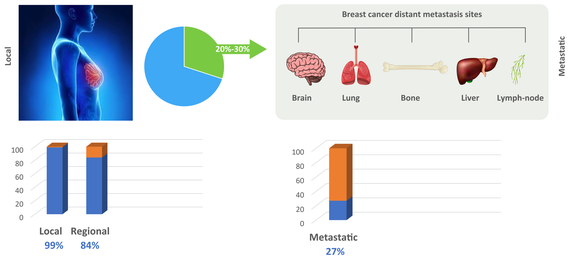
Figure 1. Different molecular subtypes of breast cancer*.



** https://www.researchgate.net/figure/Different-molecular-subtypes-of-breast-cancer_fig1_343725505*

Background - Survival

Figure 2. Background - Survival



- Even though the physiological conditions that stimulate the growth of cancer cells are not clearly understood, we know the development of malignant tumors is controlled by a complex biologic system, involving the interaction between tumor cells, stromal cells and the host's inflammatory cells.
- Checkpoint proteins regulate the immune system.
- Breast cancer cells exploit the up-regulation or down-regulation of these proteins to evade anti-tumor immune responses.
- Soluble forms of immune checkpoint molecules (ICM) can be measured in human plasma; however, their biological and clinical significance remains essentially unknown.
- The ability to reliably measure serum based immune mediators adds tremendous value in this field of research.

Methods

- The present study was a pilot study, aimed to measure the ICM in metastatic BC patients and compare them to healthy controls.
- Soluble forms of ICM were measured using Multiplex® bead array and ELISA technologies.
- Plasma samples from 20 metastatic breast cancer (MBC) patients and 45 healthy controls were analyzed for each protein.
- Data was prospectively obtained.
- Measured levels were compared between MBC patients and healthy controls using a non-parametric test (Mann-Whitney).
- *p*-values below 0.05 were considered statistically significant.

Results

Table 1. Patient Characteristics

Patient Characteristics n=20	
Age 53 (34-79)	
Neutrophil Lymphocyte Ratio (NLR)	
Median Age	3.18
Range	0.35-10.97
Metastatic Sites	**n (%)**
Liver	10 (50%)
Bone	8 (40%)
Lung	6 (30%)
Other (brain, rectal, nodal, skin)	4 (20%)
ECOG	**n (%)**
PS=0	11 (55%)
PS=1	7 (35%)
PS=2	2 (1%)
≥ 40%	45 (63%)
Unknown	1 (1%)

- The median age of the cohort was 53 years (range 34-79 years).
- The main metastatic sites included liver (10 pts), bone (8 pts), and lung (6pts), with the brain-, nodal-, rectum- and skin metastasis presenting in 1 patient each.
- The performance status was as follows; PS=0 (11 patients), PS=1 (7 patients), and PS=2 (1 patients).
- The median neutrophil-lymphocyte ratio (NLR) was 3.18 (range 0.35 – 10.97).

Table 2. Comparing the median levels of systemic soluble immune checkpoints in metastatic breast cancer patients with those of healthy controls.

			Metastatic Breast Cancer (n=20)	Controls (n=45)	
	ICM		Median pg/ml (95%CI)	Median pg/ml (95%CI)	*p* value
Co-stimulatory	CD27	DOWN	2364,87 (1214,96 - 4249,63)	4577,35 (3391,13 - 5784,85)	0,0039
	CD28	DOWN	21106,26 (13421,92 - 36668,75)	46135,18 (27210,29 - 67544,1)	0,0069
	CD40	DOWN	1285,74 (836,51 - 1924,37)	1977,68 (1404,82 - 2569,56)	0,0022
	ICOS	DOWN	16001,67 (5033,1 - 25988,1)	26506,65 (15897,52 - 31725,99)	0,0157
	GITR	DOWN	1621,68 (266,85 - 2390,84)	3797,68 (1993,96 - 5396,86)	0,0011
	GITRL	DOWN	3207,48 (1092,21 - 4795,97)	7151,12 (1092,21 - 4795,97)	0,0000
	CD86	DOWN	2930,8 (762,93 - 5579,81)	14297,09 (9391,46 - 20525,14)	0,0000
	CD80	DOWN	1833,18 (459,93 - 3030,69)	2329,77 (1395,01 - 3042,87)	0,0992
Co-inhibitory	PD-1	DOWN	13350,79 (3695,61 - 20379,62)	14917,48 (7874,92 - 21795,02)	0,2325
	PD-L1	DOWN	1616,5 (546,89 - 2807,31)	3342,62 (2628,64 - 4750,96)	0,0002
	CTLA-4	DOWN	910,96 (220,6 - 1742,48)	2618,23 (1578,44 - 3110,47)	0,0002
	TIM-3	UP	7438,2 (6430,35 - 9885,27)	5046,87 (4732,72 - 5958,87)	0,0001
	LAG-3	UP	480708,67 (245454,27 - 673316,46)	150416,02 (94508,53 - 187997,23)	0,0000
	BTLA	DOWN	12380,49 (2788,14 - 17513,4)	18147,26 (11461,86 - 25180,69)	0,0145
Dual	TLR-2	DOWN	19061,48 (10368,32 - 33291,28)	30477,2 (20928,44 - 50302,64)	0,0039
	HVEM	DOWN	2115,98 (1744,07 - 2332,1)	2290,19 (2079,46 - 2618,44)	0,0626
Other	Arginase	DOWN	25,52 (25,52 - 25,52)	78,64 (38,03 - 195,47)	0,0033
	RANTES	UP	51,95 (39,31 - 62,88)	48,72 (36,3 - 66,96)	0,4861
	TGF-β1	DOWN	5443,42 16184,42 - 36390,72	23785,83 (3613,79 - 11090,75)	0,0000

Figure 3 A & B. Comparison of plasma levels of immune checkpoints between healthy controls, early breast cancer patients and MBC patients.

Fig 3A. LAG-3

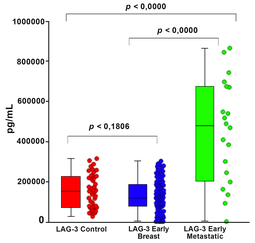


Fig 3B. TIM-3

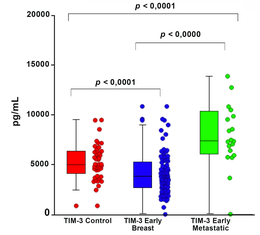


Figure 3 C & D. Comparison of plasma levels of immune checkpoints between healthy controls, early breast cancer patients and MBC patients.

Fig 3C. PD-L1

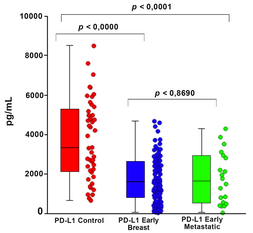


Fig 3D. CTLA-4

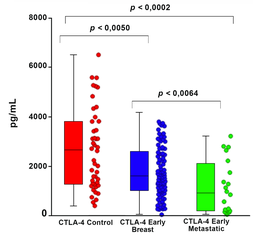


Figure 3 E & F. Comparison of plasma levels of immune checkpoints between healthy controls, early breast cancer patients and MBC patients.

Fig 3E. GITR

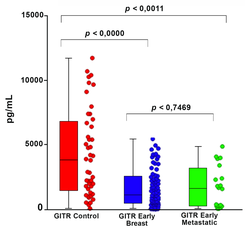
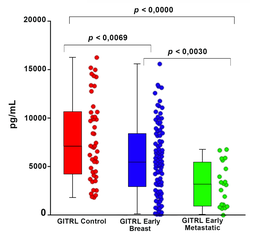


Fig 3F. GITRL



Conclusions

- With this pilot study we identified low levels of CD27, CD28, CD40, ICOS, GITR, GITRL, CD86, PD-L1, CTLA-4, BTLA, arginase, and TGF-β1, and high levels of TIM-3 and LAG-3 immune checkpoint molecules in MBC patients compared to healthy controls.
- These results indicate that a down-regulation of soluble ICM pathways and an up-regulation of some inhibitory ICM pathways are associated with MBC patients.
- To our knowledge, this is the first study to describe soluble immune checkpoint molecules in MBC patients.

Future Research

- The study will be expanded, looking at disease biology (e.g., TNBC, Her-2 status, hormone status etc.) and their correlations with responses.

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