

Dysregulation of immune checkpoint proteins in newly-diagnosed early breast cancer patients

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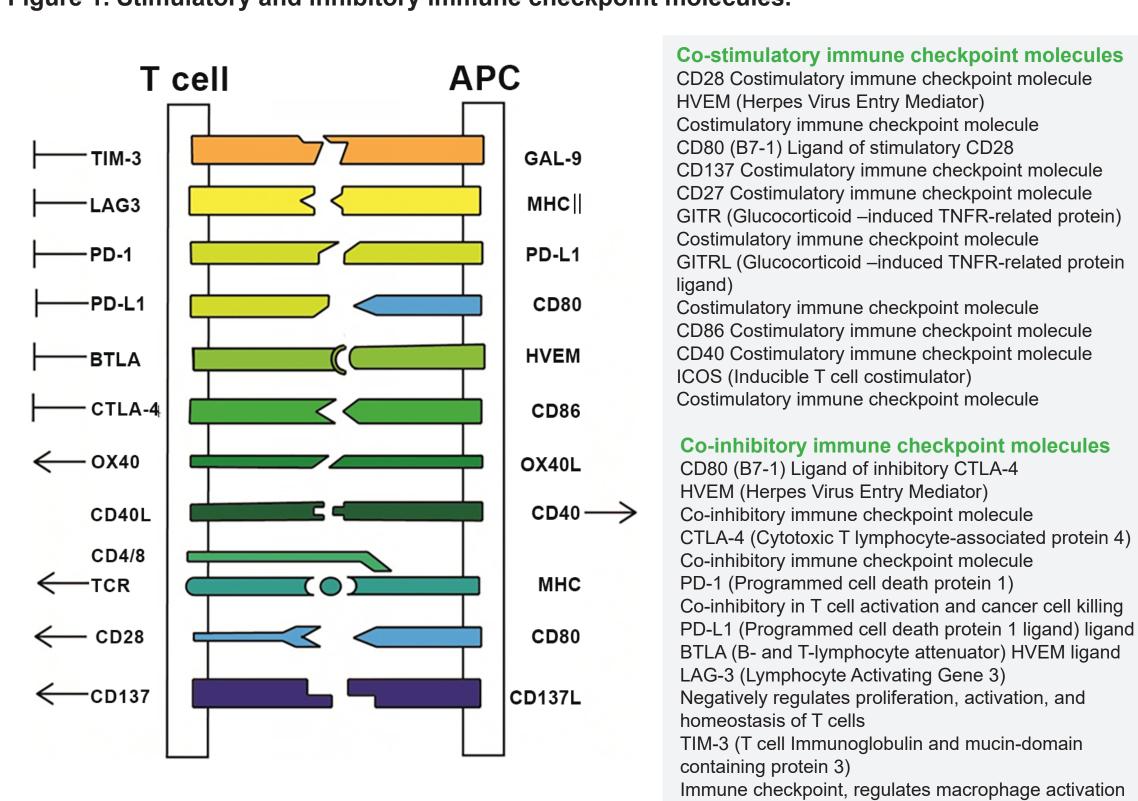


Background

summarized in figure 1;

- For effective killing of cancer cells in an anticancer immune response, a series of events involving different immune cells needs to be initiated and allowed to proceed. The steps in the cancer immunity cycle start with the release of tumor cell antigens, which are recognized by and lead to the killing of cancer cells by cytotoxic T cells. This immune response is modulated by a variety of stimulatory and inhibitory factors;
- T cells need two signals for activation: binding of the TCR (T-cell receptor) to the MHC (major histocompatibility complex) and activation of co-stimulatory molecules;
 Immune checkpoints can stimulate or inhibit these events thereby regulating the functions of immune cells;
- Accordingly, checkpoints play important roles in the maintenance of immune homeostasis;
 Examples of stimulatory molecules include TCR/MHC, CD137L/CD137 and OX40L/CD40, while CTLA-4/CD80 or CD86 and PD-1/PD-L1 are potent inhibitory checkpoints. Increasing numbers of novel regulatory receptors and ligands have recently been described and are
- ▶ Recently, a series of soluble systemic immune checkpoint molecules (ICM) such as sCTLA-4 (soluble CTLA-4), sPD-1 (soluble PD-1) and others have been identified that can be measured in plasma.

Figure 1. Stimulatory and inhibitory immune checkpoint molecules.



Reference

Gu, D., Ao, X., Yang, Y. et al. Soluble immune checkpoints in cancer: production, function and biological significance. j. immunotherapy cancer 6, 132 (2018).

Methods

AIM

▶ The circulating levels of 16 immune checkpoint-related proteins panel (BTLA, GITR, GITRL, HVEM, LAG-3, PD-1, PD-L1, TIM-3, CD27, CD28, CD80, CD86, CD40, ICOS, TLR-2 and CTLA-4) were profiled in 75 early breast cancer patients (patient characteristics are summarized in table 1) and compared to those of 45 healthy controls.

Laboratory Method

Plasma levels of immune-oncology checkpoints were assayed using Bio- Plex Suspension Bead Array platforms (Milliplex® or Bio-Rad® human magnetic bead panels). The methods were followed according to the manufacturers specifications and the data analysed using Bio-Plex Manager software 6.0 and results reported as pg/mL.

Statistical Methods

- The primary hypothesis was that that there was a significant difference in the plasma levels of soluble immune checkpoints between early breast cancer patients' pre-treatment, post-neoadjuvant chemotherapy (NAC), and post-surgery.
- ▶ Data was prospectively obtained, and levels compared between pre-treatment, post-NAC, post-surgery, and healthy controls using non-parametric tests (Mann-Whitney & Kruskal-Wallis).
- ▶ Descriptive statistics were used to tabulate patient characteristics. The Mann Whitney U-test was used to compare levels of the various test biomarkers between breast cancer patients and healthy controls. P < .05 was considered statistically significant.
- NCSS software version 11 for Windows (USA) was used for statistical analyses.

Results

eristics are show	n in table 1.			
Table 1. Patient Characteristics.		ntire patient		
Age		Pathological Complete Response (pCR)		
54	Yes	44 (61,11%)		
29-85	No	28 (38,89%)		
Menopausal Status		pCR by Biological Type		
46 (64%)	Her-2 F	Her-2 Positive		
25 (35%)	Yes	8 (80%)		
1 (1%)	No	2 (20%)		
Grade		Luminal		
1 (1%)	Yes	3 (30%)		
20 (28%)	No	7 (70%)		
49 (68%)	TN	TNBC		
2 (3%)	Yes	33 (65%)		
Tumor Size		18 (35%)		
21 (29%)	TNBC &	TNBC & Luminal		
42 (58%)	Yes	0 (0%)		
6 (8%)	No	1 (100%)		
3 (4%)				
Nodal Status				
36 (50%)				
36 (50%)				
Stage				
12 (17%)				
32 (44%)				
20 (28%)				
8 (11%)				
Biological Type				
10 (14%)				
1 (1%)				
9 (13%)				
51 (71%)				
1 (1%)				
	pe 54 29-85 sal Status 46 (64%) 25 (35%) 1 (1%) ade 1 (1%) 20 (28%) 49 (68%) 2 (3%) r Size 21 (29%) 42 (58%) 6 (8%) 3 (4%) Status 36 (50%) 36 (50%) 32 (44%) 20 (28%) 8 (11%) cal Type 10 (14%) 1 (1%) 9 (13%) 51 (71%)	response for the e cohort and by biol ge 54		

Table 3. The effect of treatments on soluble, systemic ICMs

Ki-67

≤ 14 %

15 - 39%

≥ 40%

Unknown

ICM	Control	Diagnosis (Pre-Chemotherapy)	Post-NAC (Post-NAC)	Post-surgery (Post-Surgery)		
	Median (pg/ml)					
BTLA	18147	13022	9987	12777		
CD80	2329	1678	3048	3611		
CD86	14297	11585	9922	12439		
CTLA-4	2618	1566	598	687		
LAG-3	150416	131275	464880	500133		
PD-L1	3342	1647	4794	5215		
PD-1	14917	12305	13350	15076		
TIM-3	5047	3897	9975	9615		
CD27	4577	3342	5351	5427		
CD28	46135	32914	44277	50058		
CD40	1977	1523	2030	2054		
GITR	3797	1497	4035	4434		
GITRL	7151	5886	5339	5927		
ICOS	26506	15123	26586	29746		
HVEM	2290	1865	4047	3950		
TI R-2	30477	26831	33837	37042		



Table 4. Comparison between the median pre-treatment ICM levels of t patients attaining a pCR and patients not attaining a pCR.

ICM	Pre-Treatment pCR (median pg/ml)	Pre-Treatmetn no pCR (median pg/ml)	p-value
BTLA	11158,79	20805,06	0,09381
CD80	1587,38	1758,04	0,37104
CD86	11140,02	12806,83	0,35118
CTLA-4	1567,38	1959,23	0,3447
LAG-3	123654,2	144059,1	0,33199
PD-L1	1625,73	1966,8	0,27858
PD-1	11086,85	13265,72	0,23135
TIM-3	3909,58	3422,375	0,90972
CD27	3150,51	3440,615	0,32575
CD28	31785,36	40785,61	0,24131
CD40	1440,05	1730,97	0,24132
GITR	1264,8	1566,92	0,46494
GITRL	5158,15	6925,995	0,09258
ICOS	14399,75	15777,33	0,31959
HVEM	1858,66	1802,72	0,60547
TLR-2	23846,32	30018,63	0,25684

There was no significant difference between the median pre-treatment ICM levels of the patients that attained a pCR compared to those patients that did not attain a pCR.

Conclusions

- We identified low levels of stimulatory and inhibitory ICMs in newly diagnosed, non-metastatic breast cancer patients compared to healthy controls.
- these pre-treatment abnormalities of systemic ICM levels corrected.
- Neo-adjuvant chemotherapy is associated with upregulation of sPD-L1 and most other ICMs.

▶ Following treatment, with the exception of CTLA-4, most of

- These results indicate that early breast cancer is associated with down-regulation of soluble stimulatory and inhibitory
- Newly diagnosed early breast cancer patients appear to have generalized immune-suppression independent of subtype and stage.
- To our knowledge, this is the first study to describe the effect of treatment on systemic ICMs in early breast cancer patients.