261P - Dysregulation of immune checkpoint proteins in newly- diagnosed early breast cancer patients undergoing neoadjuvant chemotherapy. A comparison between TNBC and non-TNBC patients.

Bernardo Leon Rapoport [1,2], Helen C Steel [2], Carol Benn [3], Simon Nayler [4], Teresa Smit [1], Liezl Heyman [1], Annette J Theron [2], Nomsa Hlatswayo [2], Luyanda LI Kwofie [2], Pieter Meyer [2], Ronald Anderson [2]

[1] The Medical Oncology Centre of Rosebank, Johannesburg, South Africa;

[2] University of Pretoria, Department of Immunology, Pretoria, South Africa;

[3] The Netcare Breast Centre of Excellence, Netcare Milpark Hospital, Johannesburg, South Africa;

[4] Gritzman & Thatcher, Johannesburg, South Africa

Background

- 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 summarized in Figure 1;
- 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.

Aim & Methods

- The circulating levels of 16 immune checkpoint-related protein panels were profiled in 72 early breast cancer patients (patient characteristics are summarized in Table 1) and compared to those of 45 healthy controls.
- The sICMs comprised six co-inhibitory (CTLA-4, BTLA, LAG-3, PD-1, PDL-1, and TIM-3), eight co-stimulatory (CD27, CD28, CD40, CD80, CD86, GITR, GITRL and ICOS) proteins, and the two dual-active sICMs, HVEM and TLR2.
- For the current analysis, we compared the sICM's on patients with TNBC vs non-TNBC patients.
- Ethics approval was granted by the Research Ethics Committee of the Faculty of Health Sciences, University of Pretoria. Informed consent was obtained from all patients and control participants.

Laboratory Method

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

Statistical Methods

- The primary hypothesis was 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 were 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

Table 1. Patient Characteristics

Patient Characteristics			
Full cohort (n=72)		**Non-TNBC (n=20)**	**TNBC (n=52)**
Age (years) 54 [29-85]	**33 [20-72]**	**29 [52-85]**	
Menopausal Status			
Peri-menopausal	1 (1%)	100%	0%
Pre-menopausal	46 (64%)	74%	26%
Post-menopausal	25 (35%)	64%	36%
Biological Type			
Her2 positive	10 (14%)	100%	0%
Luminal A	1 (1%)	100%	0%
Luminal B	9 (13%)	100%	0%
TNBC	52 (72%)	0%	100%
Grade			
I	1 (1%)	0	100%
II	20 (28%)	40%	60%
III	49 (68%)	22%	78%
Unknown	2 (3%)	50%	50%
Stage			
I	12 (17%)	17%	83%
IIA	32 (44%)	28%	72%
IIB	20 (28%)	35%	65%
III	8 (11%)	25%	75%

Patient Characteristics			
Full cohort (n=72)		**Non-TNBC (n=20)**	**TNBC (n=52)**
Age (years) 54 [29-85]	**33 [20-72]**	**29 [52-85]**	
Glands			
Negative	36 (50%)	19%	81%
Positive	36 (50%)	36%	64%
Estrogen Status			
Negative	53 (74%)	8%	92%
Positive	19 (26%)	84%	16%
Progesterone Status			
Negative	62 (86%)	16%	84%
Positive	10 (14%)	100%	0%
Her2 Status Status			
Negative	62 (86%)	16%	84%
Positive	10 (14%)	100%	0%
Ki-67 mean = 50% [6-100%]			
15 - 39%	23 (32%)	48%	52%
≤ 14%	3 (4%)	67%	33%
≥ 40%	45 (63%)	16%	84%
Unknown	1 (1%)	0%	100%

Median ICM levels compared between TNBC and non-TNBC patients at pre-treatment, post-NAC, and post-surgery.

Figure 1. HVEM

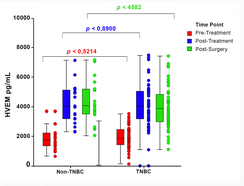


Table 2. HVEM

	Patients	Median pg/ml	CL Median 95%	*p*-value
HVEM Pre-treatment	Non-TNBC (20)	1754	1389 - 2143	0,5214
	TNBC (51)	1880	1674 - 2196	
HVEM Post-treatment	Non-TNBC (20)	4029	3218 - 5019	0,8900
	TNBC (51)	4047	3571 - 4445	
HVEM Post-surgery	Non-TNBC (20)	4076	3584 - 5172	0,4582
	TNBC (51)	3872	3350 - 4399	

Figure 2. TIM-3

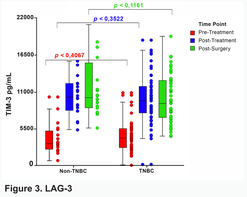


Table 3. TIM-3

	Patients	Median pg/ml	CL Median 95%	*p*-value
TIM-3 Pre-treatment	Non-TNBC (20)	3305	2757 - 549	0,4067
	TNBC (51)	4057	3145 - 4674	
TIM-3 Post-treatment	Non-TNBC (20)	10245	8642 - 12229	0,3522
	TNBC (51)	9688	8415 - 10516	
TIM-3 Post-surgery	Non-TNBC (20)	10179	8877 - 12790	0,1161
	TNBC (51)	9339	8047 - 11178	

Figure 3. LAG-3

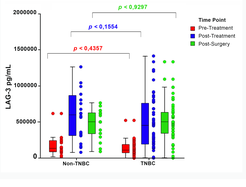


Table 4. LAG-3

	Patients	Median pg/ml	CL Median 95%	*p*-value
LAG-3 Pre-treatment	Non-TNBC (20)	139392	91752 - 225360	0,4357
	TNBC (51)	125630	91568 - 180925	
LAG-3 Post-treatment	Non-TNBC (20)	597519	324092 - 803830	0,1554
	TNBC (51)	451658	267725 - 521630	
LAG-3 Post-surgery	Non-TNBC (20)	500133	342189 - 611907	0,9297
	TNBC (51)	500133	450708 - 559532	

Figure 4. PD-L1

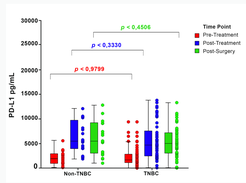


Table 5. PD-L1

	Patients	Median pg/ml	CL Median 95%	*p*-value
PD-L1 Pre-treatment	Non-TNBC (20)	1946	1091 - 2782	0,9799
	TNBC (51)	1637	1242 - 2369	
PD-L1 Post-treatment	Non-TNBC (20)	5489	3988 - 8142	0,3330
	TNBC (51)	4687	3559 - 5738	
PD-L1 Post-surgery	Non-TNBC (20)	5495	3280 - 9060	0,4506
	TNBC (51)	4953	4104 - 5969	

Figure 5. GITR

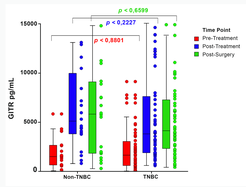


Table 6. GITR

	Patients	Median pg/ml	CL Median 95%	*p*-value
GITR Pre-treatment	Non-TNBC (20)	1431	618 - 2569	0,8801
	TNBC (51)	1602	818 - 2153	
GITR Post-treatment	Non-TNBC (20)	4922	3881 - 8394	0,2227
	TNBC (51)	3852	3666 - 5053	
GITR Post-surgery	Non-TNBC (20)	5803	1869 - 8851	0,6599
	TNBC (51)	4085	3292 - 6046	

Figure 6. CD28

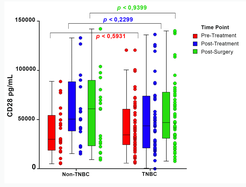


Table 7. CD28

	Patients	Median pg/ml	CL Median 95%	*p*-value
CD28 Pre-treatment	Non-TNBC (20)	30028	21202 - 49506	0,5931
	TNBC (51)	34140	29326 - 43474	
CD28 Post-treatment	Non-TNBC (20)	50298	40334 - 82883	0,2299
	TNBC (51)	43184	28182 - 51220	
CD28 Post-surgery	Non-TNBC (20)	60680	25720 - 80046	0,9399
	TNBC (51)	47413	34830 - 64706	

Figure 7. CD40

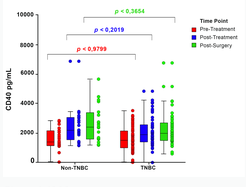


Table 8. CD40

	Patients	Median pg/ml	CL Median 95%	*p*-value
CD40 Pre-treatment	Non-TNBC (20)	1428	1226 - 2068	0,9799
	TNBC (51)	1526	1263 - 1840	
CD40 Post-treatment	Non-TNBC (20)	2212	1896 - 3040	0,2019
	TNBC (51)	1925	1648 - 2139	
CD40 Post-surgery	Non-TNBC (20)	2409	1641 - 3237	0,3654
	TNBC (51)	2009	1735 - 2323	

Figure 8. ICOS

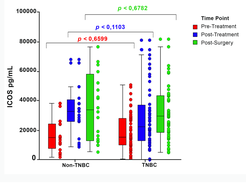


Table 9. ICOS

	Patients	Median pg/ml	CL Median 95%	*p*-value
ICOS Pre-treatment	Non-TNBC (20)	15010	10665 - 24082	0,6599
	TNBC (51)	15268	11446 - 22087	
ICOS Post-treatment	Non-TNBC (20)	32727	26231 - 38116	0,1103
	TNBC (51)	22480	19707 - 30245	
ICOS Post-surgery	Non-TNBC (20)	33841	13999 - 52000	0,6782
	TNBC (51)	29598	23673 - 33124	

Figure 6a. Association of the pre-treatment inhibitory soluble immune checkpoint PD-L1, with LAG-3, TIM-3 and HVEM.

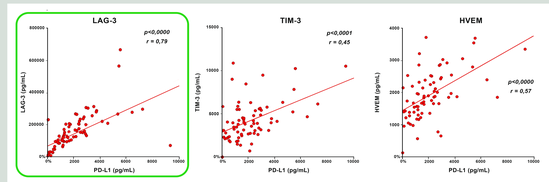


Figure 6b. Association of the post-treatment inhibitory soluble immune checkpoint PD-L1, with LAG-3, TIM-3 and HVEM.

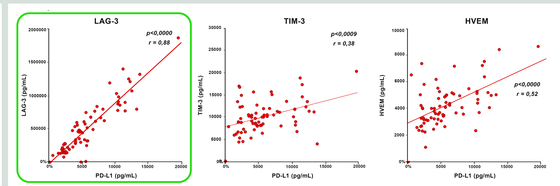


Figure 7a. Association of the pre-treatment inhibitory soluble immune checkpoint PD-L1, with LAG-3, TIM-3 and HVEM in TNBC patients.

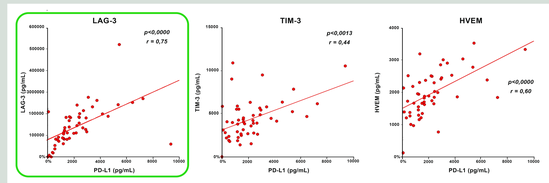


Figure 7b. Association of the post-treatment inhibitory soluble immune checkpoint PD-L1, with LAG-3, TIM-3 and HVEM in TNBC patients.

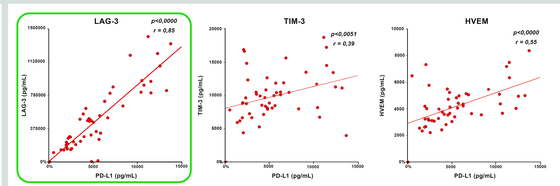


Figure 8a. Association of the pre-treatment inhibitory soluble immune checkpoint PD-L1, with LAG-3, TIM-3 and HVEM in non-TNBC patients.

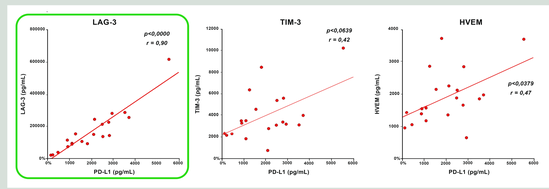
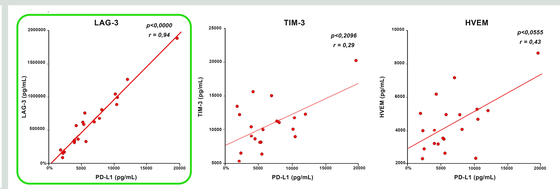


Figure 8b. Association of the post-treatment inhibitory soluble immune checkpoint PD-L1, with LAG-3, TIM-3 and HVEM in non-TNBC patients.



Conclusions

- We previously demonstrated low levels of co-stimulatory and co-inhibitory sICMs in newly diagnosed, non-metastatic BC patients.

Novel findings

- Following treatment with NAC, the sICMs levels increase substantially.
- In the case of co-stimulatory sICMs, indicative of an immune-restorative mechanism.
- The pattern of co-inhibitory sICMs (up to 4-fold elevation of PD-L1, LAG-3, TIM-3, and HVEM), might be indicative of immune-therapeutic resistance.
- There are no significant differences between TNBC and non-TNBC patients.

Future Research

- Combination approaches may be required to overcome tumor immune inhibitory mechanisms.

ESMO Congress 2023 - October 20 - 24 October, 2023; Madrid, Spain

Corresponding author: bernardo.rapoport@up.ac.za