Systemic levels of the soluble co-inhibitory immune checkpoints, CTLA-4, LAG-3, PD-1/PD-L1 and TIM-3 are markedly increased in basal cell carcinoma

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Background

- Basal cell carcinoma (BCC) is the most common malignancy, comprising about 75 % of all cases of skin cancer, and the incidence is rising[1,2].
- BCC rarely metastasizes and the mortality rate is low; however, the disease is associated with substantial morbidity[3].
- The hedgehog intracellular signalling pathway regulates cell growth, and aberrant activation of this pathway leads to BCC development[3]. The hedgehog inhibitors vismodegib and sonidegib are currently approved for systemic therapy of BCC in Europe[3,4,5].
- Hedgehog-dependent tumors are characterized by increased infiltration or the presence of suppressive immune cells, such as M2-like tumor-associated macrophages (M2-TAMs), myeloid-derived suppressor cells (MDSCs), regulatory T (Treg) cells, and cancer-associated fibroblasts (CAF)[6-10].
- BCC is associated with increased numbers of regulatory cells (Tregs) and a CAF-induced immunosuppressive microenvironment[11-14].
- Checkpoint proteins are critical for maintaining self-tolerance and modulating the immune responses of effector cells in normal tissues to minimize tissue damage. These proteins also modulate the immune infiltrates in the tumor microenvironment (TME). Cancer cells exploit the up-regulation or down-regulation of these proteins to evade the anti-tumor immune response[15,16].
- Soluble forms of immune checkpoint molecules (ICMs) have recently been identified and can be measured in human plasma; however, their biological and clinical significance remains essentially unknown[17,18]. Co-inhibitory immune checkpoint proteins are primarily involved in promoting inhibitory cell-cell interactions in adaptive immunity, especially tumor immunity.
- The soluble cell-free variants of these molecules are detectable in the circulation of cancer patients where they retain immunosuppressive activity.
- Little is known about the systemic levels of these soluble co-inhibitory immune checkpoints in patients with various subtypes of basal cell carcinoma (BCC), which is the most invasive and treatment-resistant type of this most commonly occurring malignancy.

Methods

Aim

- The study population consisted of a total of 40 South African patients (12F:28M; mean age ±SD: 69.1 ± 11.1 years) with advanced BCC attending the Dermatology Screening Clinic at Steve Biko Academic Hospital, Pretoria, South Africa.
- The cohort consisted of 40 patients with BCC, relative to those of a group of control participants (n=20).
- We measured the systemic concentrations of five prominent co-inhibitory immune checkpoints, namely CTLA-4, LAG-3, PD-1/PD-L1 and TIM-3.
- A combination of multiplex bead array, laser nephelometry and ELISA technologies were used.
- 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.

Statistical Analysis

- The primary hypothesis was that that there was a significant difference in the plasma levels of soluble co-inhibitory immune checkpoints between BCC patients and healthy controls.
- Descriptive statistics were used to tabulate patient characteristics.
- The Mann Whitney U-test was used to compare levels of the various test biomarkers between BCC patients and healthy controls.
- The area under the ROC curve (AUC) was used as a measure of discriminatory ability for the biomarkers. The Youden index, a summary measure of the ROC curve, was used as an agnostic method for choosing an optimal cut-off value on the biomarker value to illustrate potential clinical usefulness.
- A correlation matrix report was used to identify correlations between variables (or subsets of variables) within the subset, using Spearman P-values to define significance.
- A *p*-value of less than .05 was considered statistically significant.
- NCSS 2021 software for Windows (USA) was used for statistical analyses.

Results

Table 1. Numbers of patients with distinct clinical types of basal cell carcinoma (BCC).

Clinical subtype of BCC	
Adenoid	(n=1)*
Basosquamous	(n=3)
Infiltrating	(n=22)
Infiltrating with squamous differentiation	(n=4)
Keratotic	(n=1)
Micronodular	(n=2)
Nodular	(n=5)
Pigmented	(n=1)^
Superficial	(n=1)^

Numbers of patients are shown in parenthesis; *African patient; ^Asian patient

Table 2. Numbers of patients with basal cell carcinomas at distinct anatomical sites.

Anatomical site	
Cheek	(n=3)^*
Chest	(n=2)
Ear	(n=4)
Forearm	(n=4)
Forehead	(n=2)
Lower limb	(n=5)
Neck	(n=2)
Nose	(n=13)^
Shoulder	(n=1)
Temple	(n=2)
Upper anterior chest	(n=2)

Numbers of patients are shown in parenthesis; *African patient; ^Asian patient

Table 3. Comparison of the systemic concentrations of soluble CTLA-4, LAG-3, PD-1, PD-L1 and TIM-3 in patients with advanced basal cell carcinoma and control participants.

Soluble immune checkpoints (pg/mL)	Patients with advanced basal cell carcinoma (n=40)	Control participants (n=20)	*P*≤
CTLA-4	749 (326 – 1 924)*	148 (50 – 444)	0.0022
LAG-3	401 252 (4 467 – 843 050)	11 115 (635 – 528 229)	0.0184
PD-1	11 303 (3 946 – 31 514)	2 575 (500 – 9 955)	0.0002
PD-L1	1 422 (185 – 7 243)	230 (21 – 1 099)	0.0043
TIM-3	7 978 (4 956 – 10 105)	1 129 (21 – 4 842)	0.0000

Results are expressed as the median values with 25%-75% interquartile ranges in parenthesis

Table 4. ROC curve cut-off values (using Youden Index) and AUC (95% CI) for immune checkpoint molecules.

Soluble immune checkpoints (pg/mL)	AUC (CI 95%)	Cut-off point (pg/mL)	Sensitivity (TPR) %	Specificity (TNR) %	*P*≤
CTLA-4	0.7569 (0.5969 – 0.859)	≥ 324,31	75	70	0.0000
LAG-3	0.7238 (0.5653 – 0.8307)	≥ 345 396,42	70	70	0.0004
PD-1	0.7525 (0.5936 – 0.8549)	≥ 4914,80	72	65	0.0001
PD-L1	0.6813 (0.5173 – 0.797)	≥ 497,98	72	65	0.0052
TIM-3	0.8475 (0.7212 – 0.9193)	≥ 6 376,95	70	90	0.0000

Figure 1. Comparison of plasma levels of inhibitory immune checkpoints between BCC patients and healthy controls.

Figure 1a: TIM-3 levels of BCC patients vs healthy controls (p<0.00034)

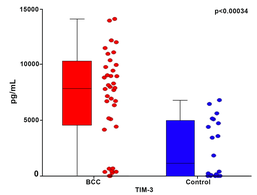


Figure 1b: LAG-3 levels of BCC patients vs healthy controls (p<0.00232)

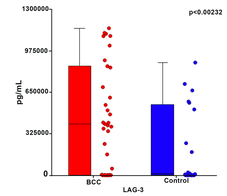


Figure 1c: PD-1 levels of BCC patients vs healthy controls (p<0.00274)

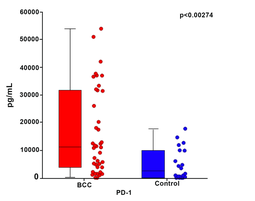


Figure 1d: CTLA-4 levels of BCC patients vs healthy controls (p<0.003)

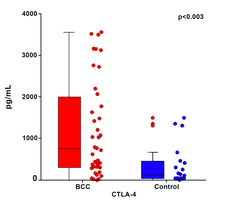


Figure 1e: PD-L1 levels of BCC patients vs healthy controls (p<0.02191)

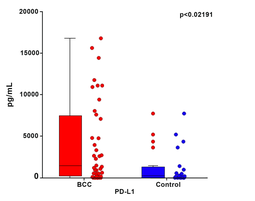


Table 5. Matrix correlation of inhibitory immune checkpoints.

Variables	CTLA-4	LAG-3	PD-1	PD-L1	TIM-3
CTLA-4	-	0.73^,* (0.00001)	0.85* (0.00001)	0.27 (0.12)	0.13 (0.47)
LAG-3		-	0.60* (0.0003)	0.50* (0.003)	0.22 (0.22)
PD-1			-	0.47* (0.006)	0.13 (0.47)
PD-L1				-	0.17 (0.36)
TIM-3					-

^The paired values represent the correlation coefficients uppermost with the corresponding P value in parenthesis.
** Denotes statistical significance*

Figure 2. ROC curves of inhibitory immune checkpoints.

Figure 2a. ROC curve of TIM-3 with AUC=0.85, confidence interval (95%): 0.72-0.92, p<0.0000).

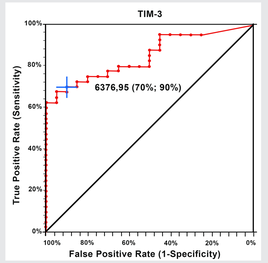


Figure 2b. ROC curve of LAG-3 with AUC=72, confidence interval (95%): 0.57-0.83, p<0.0004).

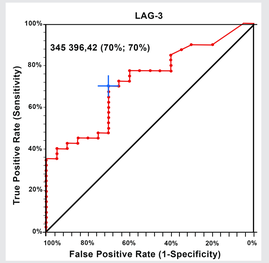


Figure 2c. ROC curve of PD-1 with AUC=0.75, confidence interval (95%): 0.59-0.85, p<0.0001).

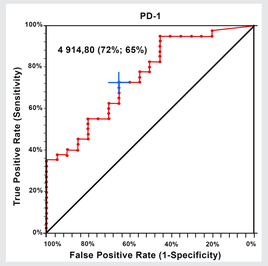


Figure 2d. ROC curve of CTLA-4 with AUC=0.76, confidence interval (95%): 0.60-0.86, p<0.0000).

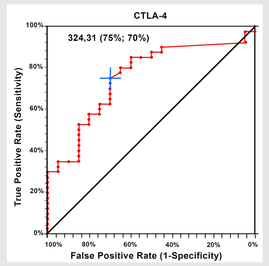


Figure 2e. ROC curve of PD-L1 with AUC=0.68, confidence interval (95%): 0.51-0.79, p<0.0052).

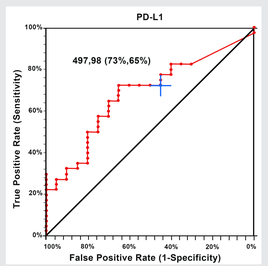
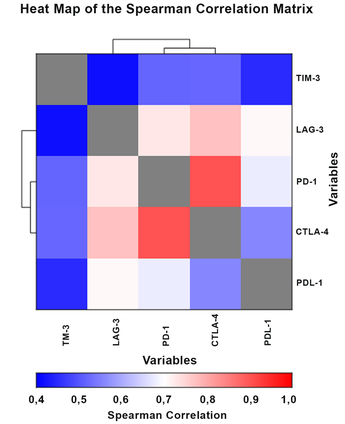


Figure 3. Heatmap of the Spearman correlation matrix



Conclusions

- **This seemingly novel finding not only identifies the existence of significant systemic immunosuppression in BCC, but also underscores the therapeutic promise of immune checkpoint targeted therapy.**
- **The study demonstrates the potential of these proteins to serve as prognostic/predictive biomarkers in BCC.**
- **The therapeutic potential of dual targeting of PD-1 and TIM-3 or LAG-3 in this condition, as well as treatment with checkpoint inhibitors early in the course of the disease, is warranted.**
- **Our current BCC research includes the investigation of the impact of soluble stimulatory immune checkpoint molecules (CD27, CD28, CD40, ICOS, GITR, GIRTL, CD86 and CD80) in this disease area.**

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