# Tumour Infiltrating Lymphocytes (TILs) in triple-negative breast cancer: High Immunoscore is associated with pathological CR in patients receiving neoadjuvant chemotherapy


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## Introduction

### Background

- Neoadjuvant chemotherapy is widely used to downstage breast cancers prior to surgery.
- Pathologic complete response (pCR) rate is a strong predictor of outcome for breast cancer.

### Immunoscore®

- The Immunoscore® assay is the first standardized immune-based assay for classification of cancer [Hermitte et al., 2016]. It assesses the host immune response by measuring intra- and peri-tumoral T cell infiltration in formalin-fixed paraffin-embedded (FFPE) tissue sections.
- Originally developed for colon cancer indication, it is intended to be widely used in solid cancer indications for diagnostic and prognostic purposes, as well as a pharmacodynamic biomarker during drug development processes. As a first clinical validation in breast cancer, we assessed the Immunoscore in a cohort of 103 breast cancer patients, that previously received neo-adjuvant chemotherapy.

## Methods

### Pathological and clinical assessment

- Clinical assessment of the primary tumour and lymph nodes was made using bi-dimensional caliper measurements of the primary tumour and axillary nodes.
- Sonographical assessments of the primary tumour and lymph nodes were performed regularly.
- Immunohistochemical staining was performed for ER, PR, HER-2 and Ki67 .
- Fluorescence in situ hybridization (FISH) was used to confirm HER-2 positivity.
- We analyzed data retrospectively/prospectively on 103 breast cancer patients undergoing neoadjuvant chemotherapy.
- Pathological complete response (pCR) was defined as the complete disappearance of the invasive cancer in the breast and absence of tumour in the axillary lymph nodes.
- Ethics approval was obtained from Pharma-Ethics, Pretoria, South Africa (ethics committee working according to the South African Ethics regulations).
- NCSS software version 11 for Windows (USA) was used for statistical analyses.
- Outcome assessments: Associations of clinical and pathological characteristics including Ki67, CD8+ cytotoxic T cells and CD3+ T cells with pCR.
- All patients were treated with anthracycline and/or taxane-based neoadjuvant chemotherapy.

### Immunoscore® Assessment

- In this retrospective analysis, 103 pre-treatment tumour tissue samples were analyzed by immunohistochemistry for density (cells/mm$^3$) of T-cell subsets (CD3+,CD8+).
- CD3 and CD8 staining was performed using Benchmark® XT station on 2 consecutive formalin-fixed paraffin-embedded (FFPE) slides (4 µm).
- Digital pathology-dedicated software permitted the measurement of positive cell densities into interest area (core of the tumour and invasive margin).
- A prespecified bioinformatics algorithm was used to generate a numerical index (Immunoscore®) and analysis cut-offs. Immunoscore® assay measures the density of CD8+ cytotoxic T cells and CD3+ T cells of resected or biopsied cancer samples and performed on FFPE tissue slides.
- Immunoscore® provides 3 score levels (high / intermediary / low).
- Immunoscore® was applied to tumours with invasive margin and was adapted when no invasion was identified on the specimen.

Figure 1. Immunoscore® Assessment.

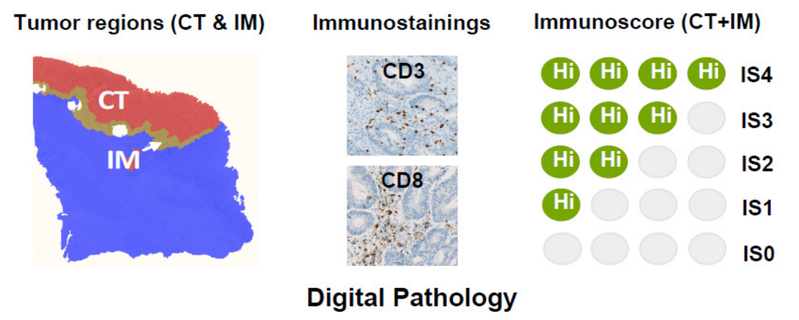



Figure 2. Immunoscore® High.

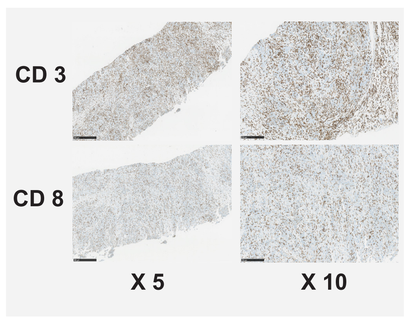



Figure 3. Immunoscore® Low.

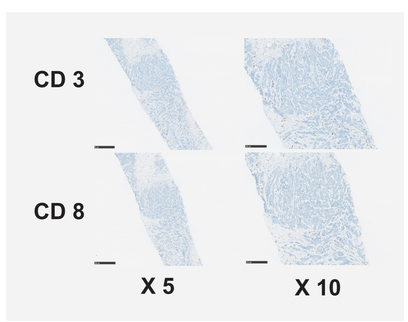
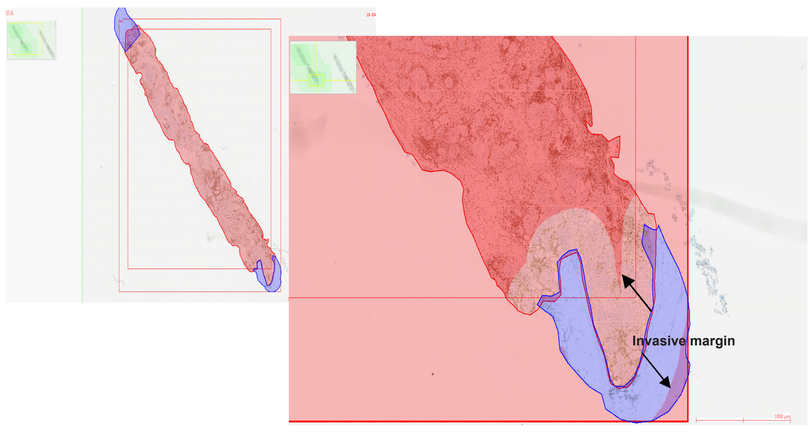



Figure 4. Invasive margin.




### Statistical Methods

- The primary hypothesis was that higher levels of CD8+ cytotoxic T cells, CD3+ T cells and Immunoscore® would be associated with a better overall prognosis, independent of anti-cancer therapy.
- The Mann Whitney U-test was used to compare the cell density between TNBC and Non-TNBC patients.
- Receiver-operating characteristic (ROC) curve analysis was used to determine the optimal cut-point for Ki67, CD8+ cytotoxic T cells, CD3+ T cells and Immunoscore®.
- Fisher's exact or Chi-squared tests were used for the analysis of categorical variables.
- Logistic regression multivariate models included only variables that exhibited a univariate association with the dependent variable, pCR (p < 0.1).
- NCSS software version 11 for Windows (USA) was used for statistical analyses.

## Patient Characteristics

Table 1. Patient Characteristics.

| | | |
|---|---|---|
| **Age (n=103)** | | |
| Median Age | 52 | |
| Range | 26-84 | |
| **Histology** | | |
| | **Total** | **%** |
| Ductal | 99 | 96% |
| Lobular | 2 | 2% |
| Other | 2 | 2% |
| **Menepausal Status** | | |
| | **Total** | **%** |
| Pre | 41 | 40% |
| Post | 62 | 60% |
| **Tumour Size** | | |
| | **Total** | **%** |
| T1 | 23 | 22% |
| T2 | 65 | 63% |
| T3 + T4 | 15 | 15% |
| **Nodal Status** | | |
| | **Total** | **%** |
| Negative | 45 | 44% |
| Positive | 54 | 52% |
| Unknown | 4 | 4% |
| **Stage** | | |
| | **Total** | **%** |
| A1 | 8 | 7% |
| A2 | 1 | 1% |
| B1 | 49 | 48% |
| B2 | 26 | 25% |
| C1 | 10 | 10% |
| C2 | 7 | 7% |
| C3 | 2 | 2% |
| **Ki-67 Pre-chemo** | | |
| Median | 40 | |
| Range | 5-90 | |
| | **Total** | **%** |
| ≥ 40% | 51 | 50% |
| 15-39% | 37 | 35% |
| < 15% | 13 | 13% |
| Unknown | 2 | 2% |
| **Molecular type** | | |
| | **Total** | **%** |
| Luminal A | 9 | 9% |
| Luminal B | 23 | 22% |
| HER2 Positive | 18 | 18% |
| TNBC | 53 | 51% |
| **CD3 and CD8 Count** | | |
| | **Median Cells/mm$^3$** | **Range Cells/mm$^3$** |
| CD3 centre of tumour | 884 | 25-5771 |
| CD3 invasive margin | 1409 | 53-6197 |
| CD8 centre of tumour | 358 | 10-3448 |
| CD8 invasive margin | 535 | 38-3117 |
| **Immunoscore** | | |
| | **Total** | **%** |
| 0 | 8 | 8% |
| 1 | 9 | 8% |
| 2 | 48 | 47% |
| 3 | 35 | 34% |
| 4 | 3 | 3% |

## Results

Figure 5. ROC Curve – CD3-Centre of Tumour.

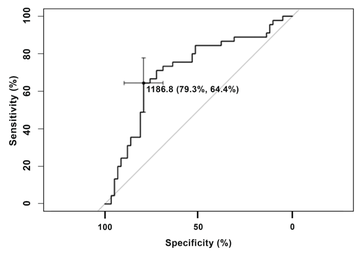



Figure 6. ROC Curve – CD8 – Centre of Tumour.

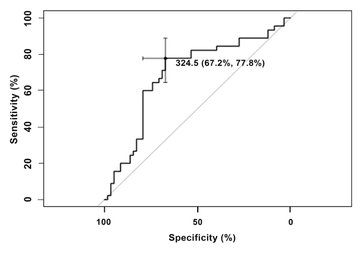



Figure 7. ROC Curve – CD3 – Invasive Margin.

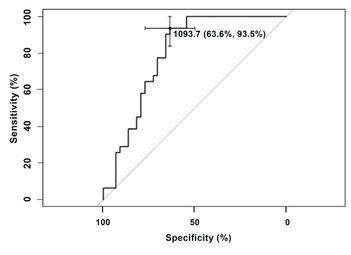



Figure 8. ROC Curve – CD8 – Invasive Margin.

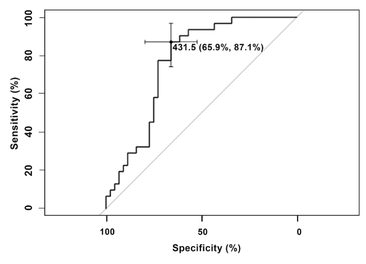



Figure 9. Percentage of patients with cell density below/over 1200 mm$^3$ (Centre of Tumour).

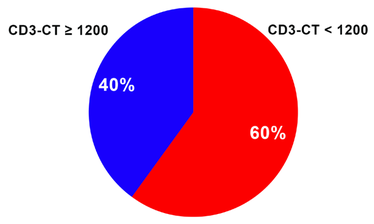



Figure 10. Percentage of patients with cell density below/over 1100 mm$^3$ (Invasive Margin).

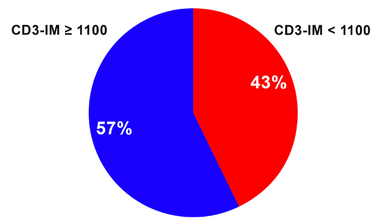



Figure 11. Percentage of patients with cell density below/over 300 mm$^3$ (Centre of Tumour).

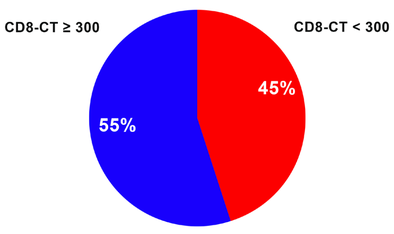
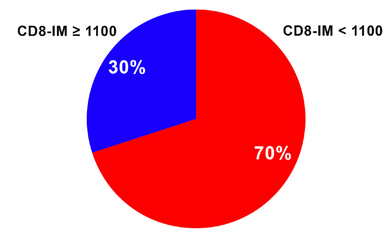



Figure 12. Percentage of patients with cell density below/over 300 mm$^3$ (Centre of Tumour).




### T-Cell densities compare between TNBC vs Non-TNBC patients

Figure 13a. CD3 - Centre of Tumour.

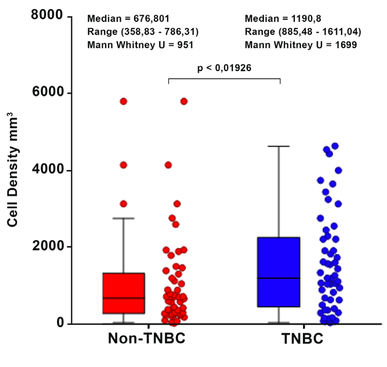



Figure 13b. CD3 - Invasive Margin.

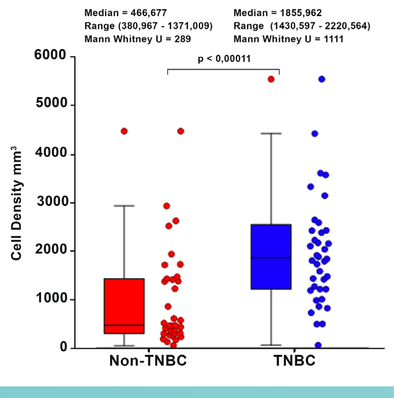



Figure 13c. CD8 - Centre of Tumour.

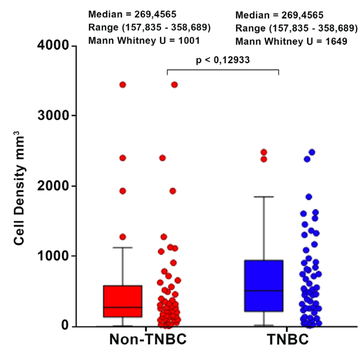
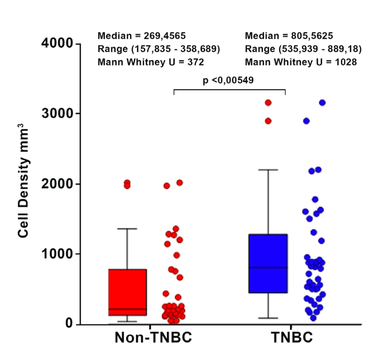



Figure 13d. CD8 - Invasive Margin.




### Univariate Analysis

Table 2. Univariate Analysis - Significant factors assosiated with pCR.

| | | | |
|---|---|---|---|
| **Stage** | | | |
| | **pCR** | **Chi²** | **pValue** |
| 1 | 67% | 9,03 | 0.02885 |
| 2A | 51% | | |
| 2B | 42% | | |
| 3 | 16% | | |
| **ER** | | | |
| | **pCR** | **Chi²** | **pValue** |
| Positive | 18% | 21.80 | 0.00001 |
| Negative | 64% | | |
| **PR** | | | |
| | **pCR** | **Chi²** | **pValue** |
| Positive | 13% | 22.81 | 0.00001 |
| Negative | 61% | | |
| **HER2** | | | |
| | **pCR** | **Chi²** | **pValue** |
| Postive | 67% | 0.3531 | 0.55237 |
| Negative | 51% | | |
| **Molecular type** | | | |
| | **pCR** | **Chi²** | **pValue** |
| Luminal | 9% | 23,03 | 0,00001 |
| HER2 Positive | 50% | | |
| TNBC | 62% | | |
| **Ki-67** | | | |
| | **pCR** | **Chi²** | **pValue** |
| ≥ 40% | 57% | 13,84 | 0,00099 |
| 15-39% | 41% | | |
| < 15% | 0% | | |
| **Immunoscore®** | | | |
| | **pCR** | **Chi²** | **pValue** |
| High | 63% | 9,99 | 0,00674 |
| Intermediate | 35% | | |
| Low | 23% | | |
| **Immunoscore®** | | | |
| | **pCR** | **Chi²** | **pValue** |
| High | 63% | 9,27 | 0,00010 |
| Intermediate + Low | 32% | | |

Figure 14. Response to Neo-Adjuvant.

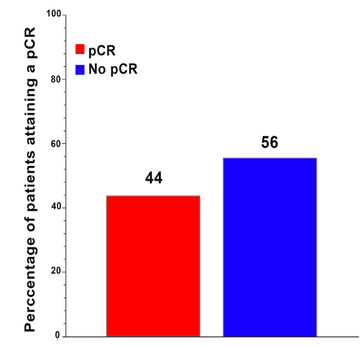
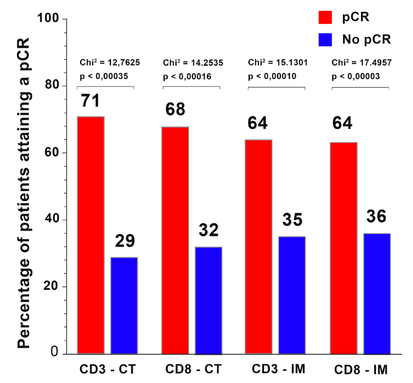



Figure 15. Percentage of patients attaining a pCR.




### Median cell density in patients with pCR vs non-pCR patients

Figure 16. Median cell density in patients with pCR vs non-pCR patients.

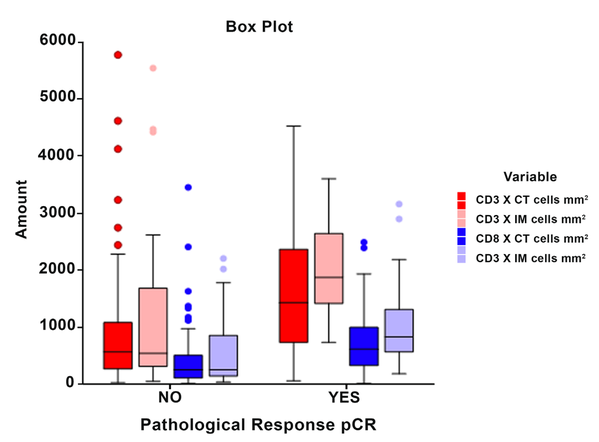
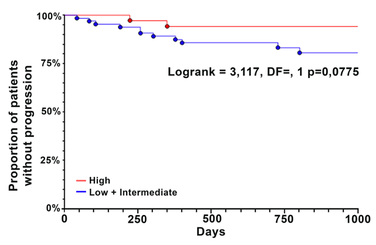



Table 3. Median cell density in patients with pCR vs non-pCR patients.

**Median cell density in patients with pCR vs non-pCR patients**

| | Outcome | Median | CI (95,0%) | p-value |
|---|---|---|---|---|
| CD3 Centre of Tumour | No pCR | 567,559 | 358,83 - 753.29 | 0,00329 |
| | pCR | 1432,01 | 1103,19 - 1900 | |
| CD3 Invasive Margin | No pCR | 540,828 | 431,97 - 1211.749 | 0,00043 |
| | pCR | 1877,745 | 1430,597 - 2418.445 | |
| CD8 Centre of Tumour | No pCR | 246,0505 | 154,086 - 307.483 | 0,01991 |
| | pCR | 614,485 | 450,177 - 749.512 | |
| CD8 Invasive Margin | No pCR | 255,148 | 175,811 - 425.343 | 0,00119 |
| | pCR | 827,267 | 643,216 - 1189.143 | |

Fiure 17. Disease free survival by Immunoscore.




### Logistic regression analysis

Table 4. Logic regression analysis.

**Coefficient Significance Tests**

| Independent | Regression Coefficient | Standard Error | Wald Z-value | Wald Prob | Odds Ratio |
|---|---|---|---|---|---|
| Ki-67 (Continuous) | 5,84051 | 1,83561 | 3,182 | 0,00146 | 343,95612 |
| Biological Type - Luminal | -2,79292 | 1,17165 | -2,384 | 0,01714 | 0,06124 |
| Immunoscore Intermediate | -1,80059 | 0,77698 | -2,317 | 0,02048 | 0,1652 |
| Immunoscore Low | -1,99918 | 0,98812 | -2,023 | 0,04305 | 0,13545 |
| Tumour 2-5cm | 2,17458 | 1,09489 | 1,986 | 0,04702 | 8,79853 |
| Biological Type - TNBC | -3,2585 | 1,66519 | -1,957 | 0,05037 | 0,03845 |
| Stage 2B | -2,58973 | 1,5177 | -1,706 | 0,08794 | 0,07504 |
| Stage 2A | -2,01162 | 1,25775 | -1,599 | 0,10974 | 0,13377 |
| Intercept | 2,63261 | 1,67336 | 1,573 | 0,11566 | 13,91008 |
| Stage 3 | -2,83108 | 1,84867 | -1,531 | 0,12567 | 0,05895 |
| ER Positive | -1,63232 | 1,72928 | -0,944 | 0,34521 | 0,19548 |
| Tumour > 5cm | -1,37975 | 1,78558 | -0,773 | 0,43969 | 0,25164 |
| PR Positive | -0,85124 | 1,1142 | -0,764 | 0,44487 | 0,42688 |

## Conclusions

- Ki-67, Biological type, Immunoscore® and tumour size are independent prognostic factors of pCR in patients with early breast cancer undergoing neoadjuvant chemotherapy.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