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ORIGINAL RESEARCH

# Transmembrane Phosphatase with TEnsin Homologue (TPTE) Expression in Epithelial Ovarian Cancer

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**Abstract**

**Background:** TPTE is a 'Cancer Testis' antigen that could be a candidate for targeted immunotherapy of epithelial ovarian cancer.
**Objective:** To determine the prevalence of expression and the impact of TPTE on clinical and survival outcomes in epithelial ovarian cancer.
**Methods:** Relevant medical information of 173 ovarian cancer patients (including Fallopian and primary peritoneal) managed at a Cancer Centre were retrieved. Reverse-transcriptase polymerase chain reaction (RT-PCR) was used to detect the expression of TPTE in the tumours. TPTE expression was correlated with the clinicopathologic and survival outcomes of the patients.
**Results:** TPTE was expressed by 45.1% (78/173) of the tested tumours. There was no significant difference in age between TPTE-positive and negative women (p = 0.93). TPTE expression was not significantly associated with the stage of the disease (p = 1.00), grade of disease (p = 0.71) and histology of the tumour (p = 0.17). There was no significant association between TPTE expression and the ease of optimum debulking (44.5% vs 44.3%, p = 0.54). TPTE expression was also not associated with a better response to therapy (p = 0.05). However, it was associated with slightly longer but not statistically significant progression-free survival (27.5 vs 20.6-months, p = 0.14) and overall survival (49.2 vs 28.0 months, p = 0.11).
**Conclusion:** This study shows that TPTE is expressed at a moderate frequency in epithelial ovarian cancers, and its expression is associated with marginally better survival outcomes.

***Keywords: Antigen-specific Immunotherapy, 'Cancer Testis' antigen, Epithelial Ovarian Cancer, ETPTE, NY-ESO-1.***

## Introduction

Ovarian cancer is a leading cause of gynaecological cancer mortality worldwide despite improvements in primary cytoreductive surgery and platinum-based chemotherapy in its management. [1, 2] It has been estimated that, in the United States, one woman in 70 will develop

ovarian cancer, and one woman in 100 will die of the disease. [3] The incidence rates of ovarian cancer vary between different countries and appear to be related to socioeconomic status and reproductive factors. It is the second most common gynaecological cancer in sub–Saharan Africa. Epithelial ovarian cancer (EOC) constitutes about 85-90% of all ovarian malignancies. [1] In current usage, the term 'epithelial ovarian cancer' refers to cancer arising from the epithelial surface of the ovary, fallopian tube and the peritoneum because of their common origin, similar presentation, and same treatment modalities. [4] In the search for novel therapeutic approaches for managing recurrent ovarian cancer, antigen-specific immunotherapy has evolved particularly against the 'cancer testis' (CT) antigens because of their restricted expression in normal tissues and variable expression in cancer cells. [5] Several CT antigens have been evaluated, and a few, such as NY-ESO-1, LAGE-1 and MAGE, are currently being studied as potential targets for immunotherapy in patients with EOC. [6-8]

Transmembrane Phosphatase with TEnsin homologue (TPTE) is a member of the CT family of antigens. It contributes to the final stages of spermatocyte differentiation in the normal testis. [9] It also shares proportionate homology with tumour suppressor protein PTEN, a phosphatidylinositol phospholipid phosphatase. [10] TPTE is also a phospholipid phosphatase located on the membrane of the Golgi complex. [9] It is speculated that TPTE might be involved in the survival of tumour cells because many tumour-derived missense mutations were observed in PTEN, resulting in cell cycle progression and inhibition of apoptosis. [11] TPTE is expressed by human cancers of the liver, the prostate, and the lungs. [11, 12] However, there is limited information about its expression in EOC. Copies of the TPTE gene are located on chromosomes13, 15, 21, 22 and Y, but only the copy on chromosome 21 appears to be expressed, and the others might be pseudo genes. [13]

The expression of CT antigens in most cancer patients naturally trigger humoral and cellular immune responses, thus producing IgG and mobilizing natural killer cells to combat these cancers. It has been demonstrated that tumours with a high population of CD4+ and CD8+ cells usually have a better prognosis. [14, 15] In a hepatocellular cancer study, TPTE was expressed in 38% of the 62 patients studied, and TPTE autoantibody was found in the sera of 25% of those that expressed the antigen. [9] In another study in patients with lung cancer, 13.4% of the 307 patients demonstrated IgG responses against TPTE in their sera. [16]

In the present study, we evaluated the prevalence of TPTE expression in EOC and assessed the correlation of TPTE expression with clinical parameters and survival outcomes.

## Methods

*Patients and specimen*

All the tissues and health record information were accessed under an institutional review board-approved protocol at the Roswell Park Cancer Centre, Buffalo, New York. All pathology specimens were examined and classified histologically according to the WHO guidelines by experienced gynaecologic pathologists. Specimens for this study were formalin fixed, paraffin-embedded and flash frozen tumour specimens obtained from patients diagnosed with ovarian, Fallopian tube and primary peritoneal carcinoma (all lumped together because of their common Mullerian origin). Health records were reviewed from a prospectively maintained database to stage each person's disease based on the FIGO 2014 Staging System, three-tiered grade, debulking status, platinum status, progression-free and overall

survival. Disease free interval is measured as the date of progression from the primary resection of the disease as evidenced by (a) twice the upper normal CA 125 (twice the peak if CA 125 never normalized), (b) biopsy-proven disease at a second-look surgery, or (c) radiological evidence of disease progression.

*Total RNA isolation*

According to the manufacturer's protocol, total RNA was isolated from frozen ovarian, fallopian, and primary peritoneal tumour tissues with Trizol TRI Reagent® (Molecular Research Center Inc, Cincinnati, OH, USA). RNA was re-suspended in RNase-free water; the concentration and purity were measured spectrophotometrically, with a cut off value of 1.6 for the 260/280 ratio. (DU Series 500 Spectrophotometer, Beckman Coulter, Fulleron, CA). The quality and integrity of the RNA were checked by electrophoresis on a 1.5% agarose gel with a formamide loading buffer.

*RT-PCR analysis of TPTE expression*

Two micrograms of each total RNA were used to generate cDNA using GE Ready-To-Go RT-PCR beads using oligo dT as the first strand primer (GE Healthcare, Buckinghamshire, UK). Testis total RNA was used as a positive control (Figure 1).

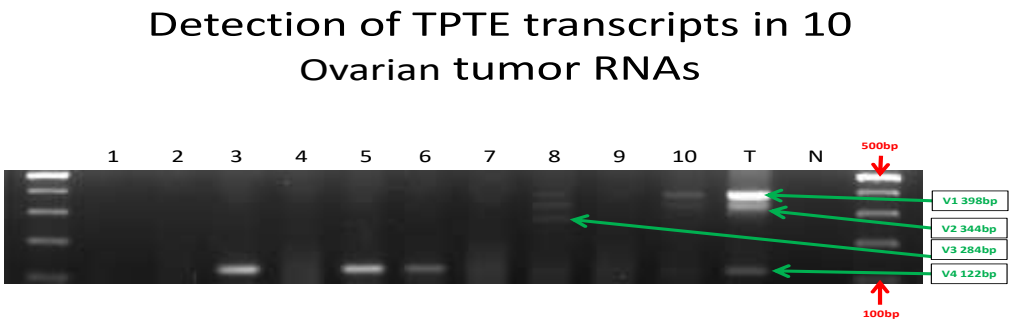



**Figure 1**. Detection of TPTE transcripts 10 ovarian tumors ( lanes 1 through 9), testis positive control (lane T) and non template control (lane N). Primers were designed to detect all 4 transcript variants of TPTE (Green arrows): variant 1 at 398bp, variant 2 at 344bp, variant 3 at 284bp and variant 4 at 122bp. Testis tested positive for variants 1, 2 and 4 while 5 of 10 tumors tested positive for variant 4. Two tumors, 8 and 10 also tested positive for variant 2, tumor 8 was the only sample to have positivity for variant 3 at 284bp.

**Figure 1: Detection of TPTE transcripts in ten ovarian tumour RNAs**

PCR primers were designed to flank or span exons on the mRNA to detect potential amplification of contaminating genomic DNA. PCR was subsequently performed to analyze the expression of TPTE. Glyceraldehyde-3-phosphodehydrogenase (GAPDH) primers were used to control for RNA integrity. PCR products were subjected to gel electrophoresis on a 1.5% agarose gel and visualized with Ethidium bromide on a UV trans-illuminator (Alpha Innotech). Bands were excised from the gel; DNA was isolated with a Quiagen mini prep kit, and samples were submitted for sequencing to verify the PCR product.

*Statistical analysis*

All statistical analyses were conducted using SAS v9.4 (Cary, NC) at a significance level of 0.05. Patient demographic and clinical characteristics were reported using the median for continuous variables and frequencies and relative frequencies for categorical variables. The association between TPTE status and disease grade or stage was evaluated using the Cochran-Armitage trend test. Overall survival and

progression-free survival were summarized by TPTE status using standard Kaplan-Meier methods, with comparisons made using the log-rank test.

## Results

*Study population*

The characteristics of the study population are presented in Table I. The median age at diagnosis of the disease was 62.3 years (range: 22.0-91.0 years). Most patients (74%) had stage III disease at diagnosis. The commonest histological type and grade (FIGO grading system) were serous (70.5%) and Grade 3 (82.5%), respectively (Table II).

*Expression of TPTE mRNA in EOC:*

The primers were designed to amplify four TEPE variants: variant 1, 398bp; variant 2, 344bp; variant 3, 284bp; and variant 4, 122bp. The expression of variants 1, 2 and 4 of TPTE was seen in normal testis, which served as a positive control (Figure 1). TPTE was expressed by 45.1% (78/173) of tested tumours (Table II).

**Table I: Baseline clinical characteristics of subjects**

| *Characteristics* | *Frequency (n = 173)* | *Percentage* |
|---|---|---|
| **Stage of disease** | | |
| I | 14 | 8.1 |
| II | 6 | 3.5 |
| III | 128 | 74.0 |
| IV | 18 | 10.4 |
| Unknown | 7 | 4.0 |
| | | |
| **Debulking** | | |
| Optimal | 119 | 68.8 |
| Suboptimal | 53 | 30.6 |
| Unknown | 1 | 0.6 |
| | | |
| **Response to therapy** | | |
| Complete response | 89 | 51.5 |
| Partial response | 2 | 1.2 |
| Persistent disease | 57 | 32.9 |
| Progressive disease | 4 | 2.3 |
| Unknown | 21 | 12.1 |

There was no significant difference in age between TPTE-positive and negative women 62.5 (37.0-86.0) years vs. 62.0 (22.0-91.0) years (p = 0.93). TPTE expression did not significantly affect the stage of disease (p = 1.00), grade (p = 0.71) and histology of the tumour (p = 0.17) (Tables III and IV).

*Correlation of TPTE expression with clinical outcome (Figure 2)*

The analysis of TPTE mRNA expression and clinicopathological characteristics are presented in Tables II and III. PTE had no significant effect on the ability to achieve optimum debulking of tumour (44.5% vs. 44.3%, (p = 0.54), no effect on response to therapy (p = 0.05), though without statistically significance, there was slightly longer progression-free survival (PFS) (27.5 vs 20.6-months, p = 0.14) and overall survival (OS) (49.2 vs 28.0 months, p = 0.11).

**Table II: Baseline pathologic characteristics of subjects**

| *Characteristics* | *Frequency (n = 173)* | *Percentage* |
|---|---|---|
| **Grade** | | |
| 1 | 11 | 6.3 |
| 2 | 18 | 10.4 |
| 3 | 142 | 82.1 |
| Unknown | 2 | 1.2 |
| | | |
| **Histology** | | |
| Serous | 122 | 70.5 |
| Endometroid | 8 | 4.6 |
| Mucinous | 7 | 4.0 |
| Clear Cell | 11 | 6.4 |
| Mixed | 1 | 0.6 |
| Non-epithelial | 10 | 5.8 |
| Other | 12 | 6.9 |
| Unknown | 2 | 1.2 |

**Table III: Demographic and clinical characteristics and survival by TPTE status**

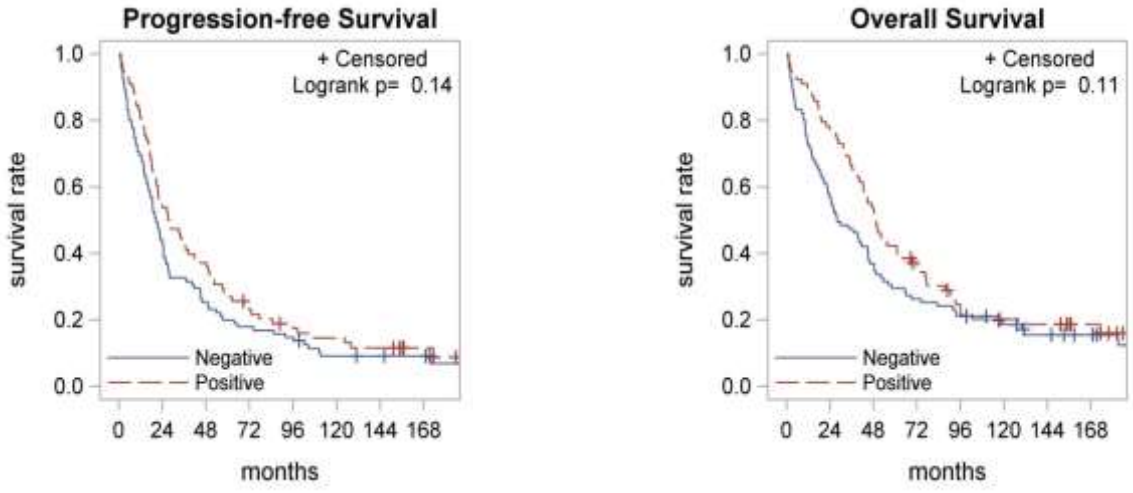
| *Characteristics* | *Expression* | | | | *P value* |
|---|---|---|---|---|---|
| | All (n = 173) | Negative (n = 95) | Positive (n = 78) | % Positive (= 45.1) | |
| **Median age (range) Years** | 62.3 (22.0-91.0) | 62.0 (22.0-91.0) | 62.5 (37.0-86.0) | | 0.93 |
| | | | | | |
| **Disease Stage** | | | | | |
| I | 14 | 8 | 6 | 42.9 | 1.0 |
| II | 6 | 4 | 2 | 33.3 | |
| III | 128 | 70 | 58 | 45.3 | |
| IV | 18 | 9 | 9 | 50.0 | |
| Unknown | 7 | 4 | 3 | 42.9 | |
| | | | | | |
| **Debulking** | | | | | |
| Optimal | 119 | 66 | 53 | 44.5 | 0.54 |
| Suboptimal | 53 | 29 | 24 | 44.3 | |
| Unknown | 1 | 0 | 1 | 100.0 | |
| | | | | | |
| **Response to therapy** | | | | | |
| Complete response | 89 | 42 | 47 | 52.8 | 0.05 |
| Partial response | 2 | 2 | 0 | 0.0 | |
| Persistent disease | 57 | 32 | 25 | 43.9 | |
| Progressive disease | 4 | 4 | 0 | 0.0 | |
| Unknown | 21 | 15 | 6 | 28.6 | |
| | | | | | |
| **Survival** | | | | | |
| PFS, median (months) | 24.1 (16.1-41.7) | 20.6 (16.1-24.6) | 27.5 (20.7-41.7) | | 0.14 |
| OS, median (months) | 38.6 (22.2-62.5) | 28.0 (22.2-44.8) | 49.2 (40.2-62.5) | | 0.11 |

**%Positive – calculated from the total in the row; PFS -Progression-Free survival; OS – Overall Survival**

## Discussion

Despite initial improvements following cytoreductive surgery and chemotherapy, most women with advanced EOC will have a recurrence. [2] Though a few other treatment modalities have evolved to circumvent this problem, nothing much has been achieved to

improve patients' survival. [17- 21] Currently, NY-ESO-1, LAGE-1 and MAGE are cancer testis antigens being evaluated as promising candidate antigens for antigen-specific immunotherapy in EOC management. [7, 8] There is a need to seek other members of the cancer testis antigen family that also have a good expression in EOC to develop multiple antigen-specific immunotherapies against the cancer to improve the survival of patients with the disease. Studies on TPTE in EOC are limited; however, it has been well studied in hepatocellular carcinoma and lung cancers. [11,16,22]




**A** **B**

**Kaplan-Meier estimates according to TPTE expression. (A) and (B) showing marginally longer PFS and OS, respectively**
**Figure 2: Survival analysis.**

**Table IV: Tumour characteristics and survival by TPTE status**

| *Characteristics* | *Expression* | | | | *P value* |
|---|---|---|---|---|---|
| | All (n = 173) | Negative (n = 95) | Positive (n = 78) | % Positive (= 45.1) | |
| **Grade** | | | | | |
| 1 | 11 | 9 | 2 | 18.2 | 0.71 |
| 2 | 18 | 6 | 12 | 66.7 | |
| 3 | 142 | 78 | 64 | 45.1 | |
| Unknown | 2 | 2 | 0 | 0.0 | |
| **Histology** | | | | | |
| Serous | 122 | 64 | 58 | 47.5 | 0.17 |
| Endometroid | 8 | 5 | 3 | 37.5 | |
| Mucinous | 7 | 6 | 1 | 14.3 | |
| Clear Cell | 11 | 4 | 7 | 63.6 | |
| Mixed | 1 | 1 | 0 | 0.0 | |
| Non-epithelial | 10 | 9 | 1 | 10.0 | |
| Other | 12 | 5 | 7 | 58.3 | |
| Unknown | 2 | 1 | 1 | 50.0 | |
| **Survival** | | | | | |
| PFS, median (months) | 24.1 (16.1-41.7) | 20.6 (16.1-24.6) | 27.5 (20.7-41.7) | | 0.14 |
| OS, median (months) | 38.6 (22.2-62.5) | 28.0 (22.2-44.8) | 49.2 (40.2-62.5) | | 0.11 |

**%Positive – calculated from the total in the row; PFS -Progression-Free survival; OS – Overall Survival**

In this study, TPTE was found to be expressed in 45.1% of EOC; this is higher than the 35% reported in an earlier study and slightly higher than the expression of NY-ESO-1, which was reported as 40.7%. [7,22] In hepatocellular cancer, TPTE expression was reported as 38%, lower than its expression in EOC as observed in the present study. [11] Cancer testis antigens expression has usually been associated with more aggressive tumours phenotypes as seen in NY-ESO-1 expressing tumours. [23-25] However, TPTE expression in the current study showed no significant difference in the stage of the disease, the grade and the histology of the tumour, suggesting that TPTE is not associated with aggressive phenotypes of EOC. This also corroborates the findings of Singhal *et al.*, which reported no significant relationships between stage, grade, and histology and TPTE expression. [22]

In hepatocellular and lung cancers, TPTE expression has been demonstrated to elicit varying degrees of autoantibody responses in positive patients' sera. Autoantibody against TPTE was reported in 25% of TPTE positive hepatocellular cancer and 13.4% of lung cancer patients. [9,14] A myeloma study also showed that 'cancer testis' antigens could activate the complement system and influence 'cancer testis' antigen processing by antigen-presenting cells, thus escalating the body's immune response to cancer. [26] This has been corroborated by studies in malignant melanoma and EOC, where autoantibody expression against NY-ESO-1 prior to immunotherapy correlated with better survival. [7 27] Even though the present study did not set out to measure the autoantibody expression in TPTE-positive EOC patients, the slightly longer progression-free survival and overall survival observed in this study may suggest that patients with TPTE expression possibly also produce autoantibody against TPTE as already demonstrated in hepatocellular carcinoma and lung cancer. Thus, there might be a role for TPTE immunotherapy in EOC management.

The present study did not show any significant difference in TPTE expression between the early stage and low-grade diseases, and the late stage and high-grade groups showed TPTE. However, it might be involved in the survival of tumour cells but not associated with the expression of aggressive EOC phenotypes.

## Conclusion

TPTE was moderately expressed in epithelial ovarian cancer in the present study, and its expression showed marginally, though not statistically significant, longer survival outcomes. As a result of this moderate expression and the antigenicity demonstrated in other cancers, TPTE might be another candidate for a more elaborate study for EOC targeted immunotherapy just like NY-ESO-1, LAGE-1 and MAGE.

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