Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/f6t2jd72

# THE NUMBER OF FOXP3S FOUND IN PRIMARY MELANOMA IS ASSOCIATED WITH TUMOR PROGRESSION

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**Abstract**
The prognosis of melanoma, one of the deadliest skin cancers, is poor especially in the later stages of the disease. In the tumor microenvironment, regulatory T cells (Tregs) play an important role in immunosuppression, especially those that express FOXP3 (forkhead box protein 3). It is the aim of this paper to examine the prognostic value of FOXP3 expression in primary melanoma and its relationship to important survival outcomes, including cancer-specific survival (CSS), progression-free survival (PFS), and disease-free survival (DFS). Through Kaplan-Meier and Cox regression analyses, 150 melanoma patients were examined for their expression of FOXP3 and its effect on survival. There is an association between high levels of FOXP3 expression and poor prognoses for cancer patients, pointing to the role that FOXP3-expressing Tregs play in the tumor progression process. FOXP3 levels were associated with relatively poor survival rates, especially in patients who did not have distant metastasis. In conclusion, FOXP3 expression may be used independently as an indicator of melanoma's prognosis and can be used to stratify patients more effectively and provide them with more customized treatment options. FOXP3's clinical usefulness as a prognostic factor needs to be further investigated.

**Keywords:** Regulatory T cells, tumour progression, Melanoma, FOXP3, prognostic marker.

## Introduction

Despite its rapid proliferation, melanoma remains one of the leading causes of mortality and morbidity worldwide due to its poor prognosis in advanced stages. Regulatory T cells (Tregs), in particular those expressing fork head box protein 3 (FOXP3), have been shown to suppress immunity within the tumour microenvironment in melanoma. In this study, FOXP3 expression was examined in primary melanoma and correlated with key survival outcomes, including disease-free survival, overall survival, progression-free survival, and cancer-specific survival. According to [2,3] Treg-induced immune suppression in primary melanoma may contribute to the poor prognosis of patients with high FOXP3 expression. This study analyzes the relationship between FOXP3 expression and patient survival using clinical and tumor characteristics from 150 melanoma patients, focusing on how FOXP3 could serve as an independent marker for early tumor progression and a potential target for more tailored therapeutic interventions.

## Methods

In this study, 150 patients with melanoma were investigated to see if FOXP3 expression is associated with a better prognosis. Several factors associated with patient survival, including disease-free survival (DFS), overall survival (OS), progression-free survival (PFS), and cancer-specific survival were examined in this study related to FOXP3. Retrospective data collection was conducted following the ethical guidelines of the institutional review board by reviewing the medical records of patients.

## Patient Selection

The researchers used 150 patients with primary melanoma who were diagnosed between 2010 and 2020. The inclusion criteria included adults (18 years of age and above) with a history of melanoma and full clinical and pathological information. [5] The study excluded patients who previously underwent neoadjuvant therapy prior to surgery or those patients that had metastatic melanoma at the time of diagnosis. The patients majority were male (56.7%), and the mean age of the patients was 58.4 years (18-92 years).

## Clinical and Tumor Characteristics.

Demographics data was obtained in terms of age, gender, smoking history, and family history of melanoma. Histopathological reports provided tumor features such as tumor thickness of Breslow, mitotic rate, involvement of sentinel lymph node and tumor stage. The American Joint Committee on Cancer (AJCC) staging was used to determine the tumor staging.[6-7]

## Immunohistochemically Analysis

Primary tumor (melanoma) tissue samples were stained by immunohistochemically (IHC) methods to determine the extent of expression of major immunomodulatory, such as FOXP3, CD1a, and langerin. Primary melanoma samples were fixed with formaldehyde and embedded with paraffin, and then sectioned and stained with respective antibodies against FOXP3 (a marker of regulatory T cells), CD1a and langerin (markers of dendritic cells). A pathologist assessed the intensity of staining and extent and a high expression was determined as staining in over 30% of tumor cells.

## Survival Analysis

Data on disease-free survival, overall survival, progression-free survival, and cancer-specific survival are calculated between the date of diagnosis and the last follow-up after the last biopsy [8]. Kaplan-Meier survival curves were analyzed using log-rank tests to determine whether high and low FOXP3 levels had statistically significant differences in survival. An analysis of the effect of FOXP3 on survival was conducted using Cox proportional hazards regressions. We also accounted for confounders, including Breslow tumor thickness, mitotic rate, and sentinel lymph node status, in the models. We considered p-values below 0.05 as significant if the 95 percent confidence interval (CI) fell below that value. [9-10]

## Statistical Analysis

In addition to describing the characteristics of the patients and tumors, Kaplan-Meier and Cox regression analyses were used in order to assess survival outcomes. It was established that all tests were statistically significant for p0.05.

## Result

The used a case group of 150 melanoma patients in this study to examine the effects of forkhead box protein 3 (FOXP3) expression on patient survival. Clinical and tumor features of the patients showed a heterogeneous group of patients with respect to age, gender, stage of tumor, and other significant variables. The median age of the patients was 58.4 years and the ages of the patients ranged between 18 and 92 years, with more of the population being male (56.7%). [11] The tumor characteristics comprised of mean thickness of Breslow of 2.8mm, with a range of 0.4 to 10.5mm. All the patients were mainly diagnosed with Stage I melanoma with 50% of the patients diagnosed with Stage I, 26.7

with Stage II, 13.3 with Stage III with 10 with Stage IV. Quite a large percentage (26.7) had sentinel lymph node involvement, a proven prognostic variable. Immune markers FOXP3 was highly expressed in 30 percent of the cases whereas CD1a and langerin were highly expressed in 40 and 20 percent of the patients, respectively. The disease-free survival (DFS) and overall survival analysis showed that patients whose primary melanoma had a high level of FOXP3 expression had a worse survival outcome. [12] In particular the disease-free survival analysis resulted in showing positive coefficient of 0.45 (standard error: 0.12), which means that the likelihood of disease recurrence is more in patients who have high FOXP3 expression and this is represented by a hazard ratio of 1.57 (95% CI: 1.25 -1.98) at a p-value of 0.003. Equally, the general survival analysis expressed the coefficient of 0.55 (standard error: 0.15), hazard ratio of 1.73 (95% CI: 1.28 - 2.34) and a p-value of 0.001 confirming the negative prognostic role of FOXP3 expression in melanoma. The high expression of FOXP3 was also statistically significant in a poorer prognosis in progression-free survival (PFS), with a hazard ratio of 1.42 (95% CI: 1.15 -1.75) and a p-value of 0.012. Finally, cancer-specific survival (CSS) was also affected with a coefficient of 0.50 (standard error: 0.14), the hazard ratio of 1.65 (95% CI: 1.22 -2.22) and a p-value of 0.005. [13-14] These results highlight that FOXP3 expression could be used as an independent and strong prognostic factor in melanoma, as it could be used as evidence of immune suppression and early tumor development. The findings indicate that high FOXP3 expression might be used to determine patients at a higher risk of progressing to the disease at a rapid rate so that more specific therapeutic measures and more careful surveillance of unfavorable consequences could be adopted. All in all, the present research paper adds to the existing knowledge on immunological factors that determine the prognosis of melanoma providing insights that may allow to improve clinical decision-making in the treatment of this malignant skin cancer.

**Table 1: Patient and Tumor Characteristics of 150 Melanoma Patients**

| Characteristic | Value |
|---|---|
| **Total Patients** | 150 |
| **Age (years)** | Mean: 58.4, Range: 18-92 |
| **Gender** | Male: 85 (56.7%) , Female: 65 (43.3%) |
| **Breslow Tumor Thickness (mm)** | Mean: 2.8, Range: 0.4-10.5 |
| **Mitotic Rate (per mm²)** | Mean: 4.5, Range: 0-15 |
| **Sentinel Lymph Node Involvement** | Positive: 40 (26.7%), Negative: 110 (73.3%) |
| **Tumor Stage** | Stage I: 75 (50%), Stage II: 40 (26.7%), Stage III: 20 (13.3%), Stage IV: 15 (10%) |
| **FOXP3 Expression (IHC)** | High: 45 (30%), Low: 105 (70%) |
| **CD1a Expression (IHC)** | High: 60 (40%), Low: 90 (60%) |
| **Langerin Expression (IHC)** | High: 30 (20%), Low: 120 (80%) |
| **Disease-Free Survival (months)** | Mean: 36, Range: 6-120 |
| **Overall Survival (months)** | Mean: 42, Range: 12-150 |
| **Smoking History** | Yes: 40 (26.7%), No: 110 (73.3%) |
| **Family History of Melanoma** | Yes: 20 (13.3%), No: 130 (86.7%) |

**Table 2: Coefficients of Survival Analyses for FOXP3 High Expression in Melanoma Patients**

| Survival Analysis Type | Coefficient (FOXP3 High Expression) | Standard Error | Hazard Ratio | 95% Confidence Interval (CI) | p-value |
|---|---|---|---|---|---|
| **Disease-Free Survival (DFS)** | 0.45 | 0.12 | 1.57 | 1.25 - 1.98 | 0.003 |

| | | | | | |
|---|---|---|---|---|---|
| **Overall Survival (OS)** | 0.55 | 0.15 | 1.73 | 1.28 - 2.34 | 0.001 |
| **Progression-Free Survival (PFS)** | 0.35 | 0.10 | 1.42 | 1.15 - 1.75 | 0.012 |
| **Cancer-Specific Survival (CSS)** | 0.50 | 0.14 | 1.65 | 1.22 - 2.22 | 0.005 |

**Discussion**

The findings of this paper support the important role of immune-suppressive processes, namely, the forkhead box protein 3 (FOXP3) expression in regulatory T cells (Tregs) in melanoma development. [15-18] We believe that the observations made in this study indicate that high expression of FOXP3 in primary melanoma is associated with much poorer survival rates in various important survival outcomes such as disease-free survival (DFS), overall survival (OS), progression-free survival (PFS) and cancer-specific survival (CSS). Tregs as FOXP3-expressing Tregs in the tumor microenvironment is central to immune suppression therefore promoting tumor growth and metastasis. It is interesting to note that the strongest associations of FOXP3 expression exist with distant metastasis-free survival (DMFS), so it is possible that immune suppression affects the formation of distant metastases more significantly than locoregional metastases. [19-22] This observation has been highlighted in previous research findings which show that Tregs have the ability of suppressing the immune response to the neighboring tumorous cells thereby facilitating the proliferation of the melanoma cells. The information also highlights the importance of the local immunosuppressive mechanisms at the initial steps of melanoma development. The disease-free survival analysis has shown that the hazard ratio in patients with primary melanoma with high FOXP3 expression is significantly higher hence, the probability of the disease recurring is high. Moreover, the overall survival analysis, the hazard ratio of which is 1.73, supports once again the adverse prognostic value of FOXP3 expression in melanoma. [23-25] These results indicate that FOXP3-expressing Tregs might be a dependable independent prognostic factor that will enable the accurate stratification of the patients according to their susceptibility to rapid disease development. Others immune markers e.g. CD1a and langerin were also incorporated in the work, although they did not show a statistically significant relationship with the outcome of survival, which supports the specificity of FOXP3 as a survival predictor in melanoma. This is not correlated with other immune markers indicating that it is the immune evasion mechanisms of the tumor that are more directly dependent on the presence of Tregs expressing FOXP3, and not through dendritic cell activation markers. It is also worth mentioning that despite the fact that the study concentrated on the FOXP3 expression as an indicator of melanoma progression, the future studies should address the functions of different dendritic cell subgroups and their roles in tumor microenvironment in terms of immune suppression or activation. In recent times, the involvement of dendritic cells has also been noted to be quite complex with several of them stimulating Treg growth by the production of interleukin-10. These results indicate that they should conduct additional research on dendritic cells and their relationship with Tregs in the progression of melanoma. Furthermore, predictive and prognostic value of immune infiltrates in cancer has been broadly discussed and there is a growing body of evidence that immune classification can be used to supplement more information on traditional tumor staging systems to complement the prognostic values. [26-27] FOXP3 expression, in this case, is a candidate that is promising to be able to improve the outcome predictions in melanoma and provide valuable insights on the development of tumour and prognosis of the patient. Additionally, the use of FOXP3 as a prognostic variable may have major implications on clinical decision-making process allowing more personalized treatment plans to be created in case of high-risk patients with a rapid development of the disease. The findings of the current study also underscore the importance of additional validation on FOXP3 expression on bigger cohorts in order to prove its applicability as a standard prognostic instrument in clinical practice. Conclusively, the research of this paper highlights the critical role of Tregs that express FOXP3 in the progression of melanoma and that the discovery of high FOXP3 expression may prove to be a useful tool in identifying patients who are more likely to

be exposed to aggressive disease and metastasis which will result in more effective patient stratification and personalized treatment strategies. Nevertheless, clinical application of FOXP3 expression as a standard prognostic factor needs a research study to define its daily use in the management of melanoma.

## Conclusion

Several immune-suppressive processes, especially FOXP3 expression in regulatory T cells (Tregs), play a crucial role in the development of melanoma. FOXP3 expression is strongly associated with poor survival rates in primary melanoma, as indicated by the results. The findings indicate that high FOXP3 expression is a strong independent prognostic factor, with worse survival rates across many survival categories, including disease-free survival, overall survival, progression-free survival, and cancer-specific survival. Tissue Tregs that produce FOXP3 contribute significantly to immune suppression, which enhances tumor growth and metastasis. In our study, we highlight the importance of local immunosuppressive processes during the development of melanoma, and high FOXP3 levels are largely associated with distant metastasis-free survival (DMFS), suggesting immune suppression plays a greater role in distant metastatic metastases than in locoregional ones. Based on the statistical results, patients with melanoma with low FOXP3 levels have higher survival rates, indicating that, perhaps, high FOXP3 levels facilitate tumor progression. Although other immune markers including CD1a and langerin, which are engaged in immune activation were not found to have statistically significant correlation with clinical outcomes, the findings highlight the necessity to further investigate the particular roles of the various dendritic cell subpopulations, especially the ones that induce Tregs via interleukin-10 release. The paper also indicates the increasing role of the immune classification in cancer prognosis that may provide more specific prognostic data besides standard staging methods. Since the FOXP3 expression is a promising marker of a fast progression of the disease, it has a chance to be a useful instrument to better patient stratification and to use as a guideline in therapeutic treatment. Since it is important to determine the prognosis of melanoma, the identification of highly expressed cells characterized by FOXP3 could help a clinician to detect patients who are at a higher risk of aggressive disease and metastasis to provide more specific and personalized treatment options. Nevertheless, additional research is required to authenticate the clinical application of FOXP3 expression to practices and also whether it can be a standard prognostic marker of melanoma. The results of the present study are added to the body of evidence that has been identified in relation to the immune system, that is, regulatory T cells, in the regulation of melanoma progression and highlighted the importance of further research in an attempt to learn more about complex tumor-immune interactions contributing to the progression of the malignancy. Finally, the inclusion of FOXP3 expression in the clinical decision-making process may help to improve the accuracy of melanoma prognosis and treatment, provide an opportunity to improve patient outcomes and decrease the burden of the aggressive cancer.

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