

DOCTORAL THESIS SUMMARY

ANALYSIS OF PROGNOSTIC MARKERS VISTA AND PD-L1 IN CUTANEOUS AND MUCOSAL MELANOMA

Doctoral Candidate: Andreea-Cătălina Tinca

Coordinator: Prof. univ. dr. habil. Ovidiu S. Cotoi

Introduction: Melanoma is an aggressive malignant tumor with a high potential for invasion and distant dissemination, originating from melanin-producing cells called melanocytes. The guarded prognosis of patients diagnosed in advanced stages and the increased risk of recurrence have led to thorough morphological, epidemiological, and prognostic investigations over time, aiming to find personalized and effective treatment options. This thesis focuses on describing the epidemiological and histological profile of our patients and investigating through an observational study two recently discovered important prognostic markers, VISTA and PD-L1.

Objectives

The purpose of this thesis was to establish the relationship between conventional diagnostic markers for melanoma, histological prognostic factors, and newly targeted prognostic markers in this pathology, specifically VISTA and PD-L1. The main objective was to investigate the specific role of VISTA and PD-L1 in melanoma, aiming to evaluate them as potential independent prognostic factors. The personal contribution to this thesis was divided into four parts, each aimed at the following objectives: identifying the epidemiological characteristics of patients, providing a detailed description of the morphological profiles of cutaneous and ocular melanoma, analyzing the immunohistochemical expression of tumors with a focus on the new markers VISTA and PD-L1, and conducting an analytical statistical study to evaluate the relationships between the described morphological and immunohistochemical characteristics and the expression of relevant markers.

General Methodology

This work aimed to conduct four independent studies on melanoma cases. Study 1 consisted of analyzing the epidemiological data of patients. Study 2 included morphological and immunohistochemical analysis of the cases. Study 3 focused on the immunohistochemical analysis of the newly investigated markers, VISTA and PD-L1. Study 4 involved statistical analysis to correlate the obtained data and interpret the relationships between the tested immunohistochemical antibodies, morphological parameters with prognostic value, and clinical case details.

Study 1 aimed to analyze the epidemiological data of patients included.

Study 1 Conclusions: In cases of cutaneous melanoma, there was an equal distribution between genders, while ocular melanoma exhibited a slight predominance in females. The mean age of patients diagnosed with melanoma was 62.6 years. The majority of cutaneous tumors were located on the dorsal region (followed by the leg, arm, and thorax). Most cutaneous melanomas were of nodular subtype, with the majority being diagnosed at stage pT4b. Regarding ocular tumors, the majority were intraocular, with exclusive choroidal involvement, classified as stage pT3a, and predominantly located at the posterior region of the eye. A total of 12 patients presented with lymph node or distant metastases, including hepatic, intestinal, and cutaneous sites. The highest number of cases was diagnosed in 2021, with the lowest incidence recorded in 2020.

Study 2 was the analysis of histological aspects and immunohistochemical expression in evaluated melanoma cases. We followed parameters necessary for reporting according to CAP (College of American Pathologists).

Study 2 Conclusions: the most frequently encountered cytological subtype in both cutaneous and ocular melanoma was the epithelioid subtype. Specific morphological features were observed: pagetoid migration was present in all cases of superficial melanoma, while ulceration was identified in over half of the nodular melanoma cases. Nodular melanoma was associated with significant morphological prognostic factors, including perineural invasion, lymphovascular invasion, and metastasis. The diagnostic markers used in our study (SOX10, S100, Melan A, HMB45) consistently showed positivity. We identified two rare melanoma types: rhabdoid

differentiation (one case) and nevoid melanoma (two cases). Capsular nevus is a relatively rare entity that can be encountered in patients with suspected lymph node metastasis from melanoma, posing a diagnostic pitfall. In cases of ocular melanoma with choroidal involvement, it is essential to assess specific features such as the number of macrophages and vascularization. Detailed quantification requires immunohistochemical reactions for the CD68 antibody (macrophage marker) and CD34 antibody (blood vessel identification). An extremely rare case diagnosed and included in the study was conjunctival melanoma, confirmed through immunohistochemical methods.

Study 3 Objectives: Analysis of the immunohistochemical reactions of prognostic markers VISTA and PD-L1. We identified the cells that were marked, types of melanoma presenting positive staining, and we quantified the cells with positive expression.

Study 3 conclusions: Immunohistochemical reactions for VISTA were positive in 65 cases of cutaneous melanoma, of which 56 were of the nodular type. The H-VISTA score indicated high expression in 41 cases of cutaneous melanoma, while low expression was observed in 24 cases. In ocular melanoma, VISTA immunostaining was detected in 8 cases, 3 of which exhibited a high H-VISTA score, and 5 showed low H-VISTA expression. The marker was positive in inflammatory cells, including macrophages and lymphocytes, as identified through routine staining and IHC techniques. Only one case of ocular melanoma, of the spindle cell type, demonstrated positive staining in tumor cells. Immunohistochemical reactions for PD-L1 were positive in 23 cases of cutaneous melanoma, all of which were nodular melanomas. The PD-L1 staining was present in T lymphocytes.

The aim of study 4 was to perform a statistical analysis that would integrate the data obtained from the morphological and epidemiological studies, with those resulting from the immunohistochemical analysis of the VISTA and PD-L1 markers. This approach aimed to elucidate the correlations between the different aspects of our study, and provide important insights into the role of these immunological markers in the clinical-pathological context of patients.

Conclusions of study 4: The statistical analysis of cutaneous melanoma highlighted that positive immunohistochemical staining for VISTA was associated with advanced tumor stage, increased Breslow index, higher Ki67 tumor proliferation index, and elevated mitotic count. A high H-VISTA score was correlated with older age and more advanced morphological features, while a low H-VISTA score was more frequently associated with superficial spreading melanoma cases, which had a lower Breslow index. Positive PD-L1 immunostaining in cutaneous melanoma was similarly associated with older patient age and more advanced morphological characteristics, reflecting the same trends observed with VISTA analysis. Comparative statistical analysis revealed significant differences between groups of patients who were VISTA positive and PD-L1 negative, and vice versa. In both cases where the markers showed positivity, the clinico-morphological profile was more advanced compared to the negative patient groups. No significant differences were demonstrated between patient groups positive for both VISTA and PD-L1.

General conclusions: The most common localization of cutaneous melanoma was at the dorsal level, most cases male patients, in the pT4b stage, followed by pT1a. Microscopically, nodular melanoma with epithelioid cells was the predominant subtype. Rhabdoid melanoma, nevoid melanoma, conjunctival melanoma and capsular nevus were rarely diagnosed cases of particular importance for differential diagnosis. VISTA immunostaining was positive in the tumor microenvironment in macrophages and T lymphocytes, both in cutaneous and ocular melanoma. PD-L1 immunostaining in the tumor microenvironment was identified in T lymphocytes and was present exclusively in nodular cutaneous melanoma. The positive marking for both VISTA and PD-L1 was associated with an advanced clinical-morphological picture of tumors and abundant inflammatory infiltrate. There are significant differences between tumors that express VISTA and do not express PD-L1, respectively tumors that express PD-L1, but are negative for VISTA. Both situations were associated with an advanced clinical-pathological picture and an increased risk of metastasis. We did not identify significant differences between VISTA-positive tumors and PD-L1.