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MELANOCYTIC TUMOUR PATHOLOGY

Tumour-infiltrating lymphocytes in melanoma prognosis and cancer immunotherapy

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Summary

The field of systemic cancer therapy for metastatic disease has entered an exciting era with the advent of novel immunomodulatory strategies targeting immune checkpoints. At the heart of these promising efforts are the tumour-infiltrating lymphocytes (TILs). As the reports demonstrating efficacy of modulating TIL effector function in patients with advanced stage cancer continue to accrue, it has become essential to better understand TIL immunobiology in order to further improve clinical outcome. In addition to providing an overview of the current immunotherapies available for metastatic melanoma, this review will briefly introduce the history and classification of TILs. Moreover, we will dissect the multifaceted roles of TILs in tumour-specific immunity and melanoma immune escape. The significance of TILs in melanoma prognosis and cancer immunotherapy will also be discussed, with a particular focus on their potential utility as biomarkers of patient response. The goal of personalised medicine appears to be in realistic sight, as new immunomodulatory techniques and technological innovations continue to advance the field of cancer immunotherapy. In light of recent studies highlighting the possible utility of TILs in determining therapeutic outcome, further characterisation of TIL phenotype and function has the potential to help translate individualised care into current medical practice.

Key words: Tumour-infiltrating lymphocyte; TIL; melanoma; immunotherapy; tumour immune escape; immunomodulation; checkpoint blockade; PD-1; CTLA-4.

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INTRODUCTION

First described by Robert Virchow in 1863, leukocytes found within tumours were initially thought to be the cells of origin of cancers arising at sites of chronic inflammation.[1] Early studies appeared to suggest that these leukocytes propagated tumour growth via protumorigenic inflammatory processes.[1] However, it was not long before it was recognised that these immune cells were often recruited as a defense mechanism against the tumour and that their presence frequently correlated with a favourable prognosis in patients with melanoma as well as other solid tumours, such as ovarian[2] and colorectal carcinomas.[3]

In 1969, Wallace Clark applied the term, 'tumour-infiltrating lymphocytes', to refer to these immune cells deployed as part of the host response to cancer.[4] In order to be regarded as TILs, lymphocytes must be in direct contact with tumour cells (RGP) and/or infiltrate tumour nests. In melanoma, Clark and Mihm reported that TILs were recruited in the radial growth phase (RGP) and led to partial regression of the primary tumour.[4] Consistent with these findings, *in vitro* studies by Hersey demonstrated cytolytic activity of these lymphocytes against autologous tumours in melanoma patients.[5] Further studies supporting the benefit of TILs revealed that their presence in the invasive vertical growth phase (VGP) of primary melanomas was associated with increased survival and decreased risk of metastasis.[6,7] Moreover, the presence of TILs in metastatic lesions appeared to also have a similar benefit. Patients with a more brisk T-cell infiltrate in lymph node metastases had improved survival rates compared with those with less reactive TIL responses.[8] Consistently, adoptive transfer of autologous TILs in combination with interleukin-2 (IL-2)-based immunotherapy has resulted in tumour regression in patients with metastatic melanoma.[9]

Over the past several years, TILs have been identified in the primary tumour, tumour-bearing lymph nodes, and visceral metastases of numerous cancer types. Given their ubiquitous nature across a vast collection of cancers in conjunction with their anti-tumour capabilities and potential roles as prognostic indicators, which will be discussed in more detail below, TILs have been garnering much deserved attention among cancer researchers as prime targets for cancer immunotherapy. However, TILs are a heterogenous group comprised of not only effector T cells, but also of tolerogenic or T regulatory (Treg) cells, functionally exhausted T cells, natural killer (NK) cells, macrophages, dendritic cells (DCs), myeloid-derived suppressor cells (MDSCs), and other immune cell types.[1] Thus, better understanding of TIL phenotype and function in the context of tumour-induced and other factors within the tumour microenvironment (TME) is crucial in aiding the invention of innovative drugs and identifying the patients most likely to respond to therapy.

CLASSIFICATION OF TILs IN MALIGNANT MELANOMA

The assessment of the role of TILs in prognostication and therapy in melanoma first required the establishment of a graded system that categorises the nature and extent of TIL

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involvement within a tumour. In 1989, Clark and colleagues[6] put forth the first comprehensive classification system defining distinct lymphocytic infiltrate patterns in melanomas (Fig. 1). They classified TILs as 'absent' when no lymphocytes were present or when the lymphocytes did not appose the melanoma cells.[6] 'Non-brisk' described a focal TIL infiltrate, while 'brisk' described either an infiltrate at the base of the VGP of the tumour or a diffuse infiltration of the entire VGP of the melanoma lesion.[6] Clemente *et al.*[7] further elaborated on these definitions and proposed that the two main patterns of a 'brisk' infiltrate be appropriately termed 'peripheral' and 'diffuse', respectively (Fig. 1A). They stressed that the term 'brisk' specifically applied to situations in which the lymphocytes were diffusely interposed between tumour cells, surrounding and disrupting them, with evidence of surrounding melanoma cell necrosis. Clemente *et al.* also defined a 'non-brisk' pattern to include a patchy multifocal lymphocytic infiltrate throughout the VGP and an infiltrate occupying one-third to one-half of the base of the VGP (Fig. 1B). Moreover, patterns such as a dense band of infiltration around the VGP but not within the tumour, a perivascular lymphocytic infiltrate, or a lymphocytic infiltrate confined to areas of fibrosis, should be given an 'absent' grade (Fig. 1C). In cases in which there are multiple tumour nodules, the overall TIL grade should be determined by the grade of the nodule with the lowest number of TILs (Fig. 1C). Clemente *et al.*[7] also noted that the 'non-brisk' grade may refer to multifocal fascicles of TILs, or to TILs that infiltrate up to one-half of the VGP (Fig. 1B). To date, the Clark classification system of TILs remains the most widely accepted method of quantifying TILs. This is most likely attributed to the ease with which this classification scheme can be taught, and to its reproducibility. A recent study instructed six observers, three dermatologists and three pathologists, on utilising the Clark classification system, and reported good interobserver agreement (kappa score > 0.6).[10]

In 2010, Rao *et al.* proposed to further subcategorise TILs based on their density (grades 1–3) and localisation pattern (focal, multifocal, and segmental) in stage T4 (>4.0 mm) primary melanomas.[11] However, this proved to have little use as neither TIL density nor TIL location correlated with survival.[11] Recently, the Melanoma Institute Australia (MIA) proposed a new grading system for TILs consisting of grade 0 through grade 3, based on their density (mild, moderate or marked) and distribution (focal, multifocal or diffuse) in the dermis (Table 1). Grade 0 described an absence of TILs; grade 1 consisted of a mild or moderate focal or mild multifocal infiltrate; grade 2 described a marked focal, a moderate or marked multifocal, or mild diffuse infiltrate; and grade 3 a moderate or marked diffuse lymphocytic infiltrate. When this classification system was applied to 1865 patients with a primary melanoma greater than or equal to 0.75 mm in thickness, a statistically significant inverse correlation was noted between TIL grade and the Clark level of melanoma invasion, number of mitoses, tumour thickness, and SLN status.[12] Moreover, the TIL grade was found to be an independent predictor of melanoma-specific survival ($p < 0.001$).[12]

While the Clark classification system for TILs remains relevant 25 years after its inception, the advent of immunotherapy targeting TIL immunobiological functions, the efficacy of which may be measured by TIL activity, has led some investigators to attempt further refinement of the current proposed classification scheme. For example, there may be greater value in a system that applies reproducible numerical values to denote TIL density, as opposed to utilising nominal categories that may be subject to interobserver variability. To our knowledge, one such classification system is currently under study and may be made available for use in the near future.

TILs AS A PREDICTIVE AND PROGNOSTIC BIOMARKER

The accurate prediction of patient prognosis is paramount to selecting the appropriate therapies and improving management of melanoma patients. Evidence to support the presence of TILs as a positive prognosticator in melanoma patients has been steadily amassing. Many studies focused on both the density of TILs and their location in relation to the tumour. In a retrospective study including 669 melanoma patients, Larsen and Grude noted improved survival rates in patients with a dense lymphocytic infiltrate seen in their primary tumours.[13] Shortly after, in 1981, Day *et al.* reported that patients with a moderate to marked infiltrate of lymphocytes within primary cutaneous melanomas had a significantly better prognosis than those with sparse or absent TILs.[14] Clark *et al.* later demonstrated that the briskness of TIL response correlated with prolonged disease-free and overall survival only in the setting of the VGP but not the RGP.[6] The 8-year survival rate was 88% in patients with a dense infiltrate of lymphocytes in their melanomas, 75% in patients with a non-brisk TIL response and 59% in patients with absent TILs.[6] Similarly, Clemente *et al.* reported a 77% 5-year survival rate in melanoma patients with a brisk TIL response compared to 53% in those with a non-brisk response and 37% in those with absent TILs.[7] In both of these studies, TIL response was an independent prognostic indicator.

On the other hand, some studies did not reveal a significant association between TILs and melanoma survival rates.[15–17] This may relate to the predominant inclusion of thin melanomas in the RGP, during which there is little support for a role of TILs in predicting outcomes, or to the potential misclassification of lymphocytes found in the vicinity of the tumour as TILs.[16] However, there are studies that have demonstrated that TILs may also be a significant prognosticator in the VGP of thin melanomas less than 1 mm in thickness.[18,19] Overall, many of the studies on TILs appear to support their relevance in melanoma prognosis.[20–22] In addition to the aforementioned studies on TILs in primary cutaneous melanomas, Mihm *et al.* found that a brisk pattern of TILs in lymph node melanoma metastases were similarly associated with an improved 5-year disease-free survival compared to a non-brisk or absent TIL pattern.[8]

As mentioned previously, it was recently shown by MIA investigators that TIL grade inversely correlates with incidence of SLN metastases.[12] This study, which represents the largest of its kind aimed at analysing the prognostic significance of TILs in melanoma, also revealed that TIL grade, independently of SLN positivity, predicted melanoma-specific survival as well as recurrence-free survival in patients with cutaneous melanoma greater than or equal to 0.75 mm in thickness.[12] The survival rate was 100% in patients with TIL grade 3 melanomas.[12] Though this study is the only study to date that demonstrates an association of TILs with all three outcomes (SLN status, recurrence-free, and melanoma-specific survival), other studies have