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MELANOCYTIC TUMOUR PATHOLOGY

Regression in cutaneous melanoma: histological assessment, immune mechanisms and clinical implications

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Summary

Tumour regression is an immunologically driven process that results in complete or partial disappearance of tumour cells. This can be observed in histological sections as replacement of tumour cells with fibrosis, angiogenesis, and a variable inflammatory infiltrate. In primary cutaneous melanoma, the prognostic significance of regression has been debated for decades, in part because inconsistent histological criteria are used in prognostication studies. It is broadly accepted that CD8+ T lymphocytes are the primary effectors of the anti-tumour response, but the interplay between melanoma and the immune system is complex, dynamic, and incompletely understood. Sustained progress in unravelling the pathogenesis of melanoma regression has led to the identification of therapeutic targets, culminating in the development of immune checkpoint inhibitors for the management of advanced disease. Modern techniques allow for high-resolution spatial analyses of the tumour microenvironment. Such studies may lead to better understanding of the immune drivers of melanoma regression, thereby facilitating the search for new prognostic and predictive biomarkers to assist clinical decision-making.

Keywords: Regression; tumour infiltrating lymphocytes; TILs; primary cutaneous melanoma; pathology; prognosis; immunology; immunotherapy; biomarkers.

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INTRODUCTION

Cancer is underpinned by a complex network of interactions between tumour and host.[1] One important but poorly understood aspect of this process is the spontaneous disappearance of tumour cells, referred to as tumour regression. In primary cutaneous melanoma, a disease known for its poor prognosis in advanced stages, the significance of regression has been investigated from a number of standpoints during the past several decades, generating a lively and oftentimes contentious discourse in the scientific literature. The progressive accumulation of clinical, pathological, and immunological data, as well as technological advancement, have led to important breakthroughs in our understanding of regression in melanoma that have informed the pathobiology, prognostication, and management of this unpredictable disease.

The current review summarises the pathological assessment, mechanisms, and prognostic implications of histological regression in primary cutaneous melanoma. This is followed by a brief discussion of its relevance to management of advanced disease, as well as new frontiers and avenues for future research.

HISTORICAL ASPECTS

The phenomenon of spontaneous tumour regression has been observed by physicians for centuries. In the 1890s, the sarcoma surgeon William Coley noticed that post-operative infection occasionally halted the progression of malignant disease.[2] He hypothesised that bacterial toxins induced a type of anti-tumour resistance. He went on to trial inoculation of cancer patients with killed *Streptococcus*, with variable success, in what is widely believed to be the first example of cancer immunotherapy.[3] Almost a century later, Everson published a review of 130 cases that he believed to be well documented examples of cancer regression, where regression was defined as the partial or complete disappearance of a malignant tumour in the absence of adequate treatment.[4] Several possible mechanisms were proposed for this phenomenon, including 'fever and/or acute infection' and 'allergic or immune reaction'. In that cohort of cases, cutaneous melanoma was the fourth most common malignancy to undergo spontaneous regression.

Regression in melanoma was first documented in 1953 by Sumner, who described a 30-year-old pregnant woman with widely metastatic disease including to the left inguinal lymph nodes.[5] Pathology of the inguinal dissection specimen showed melanoma metastases in some nodes whilst others revealed only fibrosis, an appearance interpreted as tumour regression. Since that time, there have been a number of reports of melanoma regression in both primary lesions and metastases. In some cases, metastases occurred in the absence of residual melanoma cells at the purported primary site, suggesting complete regression of the primary tumour.[6] These observations, as well as the finding of a higher frequency of melanoma in immunosuppressed patients,[7] led to the notion of an immune-mediated host response and subsequent trials of immune therapies in the management of advanced disease.[8] Indeed, melanoma is now considered a paradigm of the

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immunogenic tumour, a property underscored by the success of modern immunotherapy approaches.[9]

HISTOPATHOLOGICAL ASSESSMENT OF REGRESSION

The prevalence of regression in cutaneous melanoma varies depending on the criteria used; the range of 10–35%[10] is frequently quoted in the literature. Clark *et al.* outlined the first set of criteria for regression, and most modern definitions are variations of this original definition.[11] According to the College of American Pathologists (CAP), characteristic histological features of regression are replacement of tumour cells by lymphocytic inflammation, attenuation of the epidermis, and non-laminated dermal fibrosis with inflammatory cells, melanophagocytosis, and telangiectasia.[12] The fibrosis encountered in regressed lesions should be distinguished from scarring resulting from local superficial traumatisation, non-regressive stromal response, or prior biopsy, although this distinction may be difficult in practice.[13]

The disappearance of tumour cells may be partial or complete. In partial regression, residual melanoma cells may be seen surrounding or within the area of fibrosis; in some cases, the entire invasive component has regressed while an overlying *in situ* component remains. Some authors make a distinction between partial and segmentary regression, where the former describes residual tumour cells admixed with regressive fibrosis, while the latter refers to cases where part(s) of the lesion show complete absence of tumour cells.[14]

Complete regression, reported to occur in <0.27% of cases,[15] is defined by total absence of melanoma cells, in which case the diagnosis is inferred from the finding of fibrosis and melanophages, a documented clinical lesion previously present at the site, and/or the presence of metastatic disease. Completely regressed melanoma can manifest as tumoural melanosis, a term describing nodular aggregates of melanin-laden macrophages and extracellular melanin in the absence of melanocytes.[16] Whilst melanin can be found in the dermis in a range of conditions, including regressing pigmented keratinocytic lesions and post-inflammatory pigmentation, dense expansile accumulation of melanin favours tumoural melanosis.

The scheme outlined above is the one most commonly used by pathologists today. However, considerable variability continues to permeate the literature.[17] One source of controversy is the stratification of regression into distinct temporal phases, which may co-exist to varying degrees in any given tumour. The first three-tier classification was proposed by Kang *et al.* in 1993.[18] In this system, early regression is represented by tumour-infiltrating lymphocytes (TILs), which disrupt and percolate between groups of tumour cells, but without any identifiable fibrosis (Fig. 1A). Intermediate regression is characterised by early fibrosis and chronic inflammation with prominent vascularity and melanophages (Fig. 1B). Late regression is described as a marked diminution of tumour cells and their replacement with dense or extensive paucicellular fibrosis with effacement of epidermis (Fig. 1C).[18] The authors conducted an interobserver concordance study using two independent assessors. They found that stratifying regression into three phases resulted in a concordance rate of 86%, compared to 96% when only the presence or absence of regression was assessed. They concluded that this approach was impractical and that simpler, more reproducible criteria were required. Subsequently, Requena *et al.*[19] addressed this issue by introducing a two-tier system, where early phase regression is represented by focal reduction of tumour cells associated with an inflammatory infiltrate but excluding fibrosis, and established regression was defined as focal disappearance of melanoma cells or area of evident fibrosis with or without other features.

Despite these early controversies, no universally accepted scheme exists today. A practical approach is to define two phases of regression: an intermediate phase characterised by immature fibrosis, and a late phase characterised by mature fibrosis.[20] TILs are then considered and graded in a separate category, recognising that in some cases, they may herald the initiation of an effective immune response. Melanoma *in situ* (without an invasive component) may also undergo regression, with distinctive dermoscopic features;[21] however, there are no histological criteria specifically addressing this scenario. In general, if regressive dermal fibrosis is found associated with an *in situ* melanoma, it may be prudent to examine deeper levels to rule out the possibility of a small invasive component.[22]

Finally, quantifying regression remains a controversial topic. A number of methods may be used, although no clear consensus exists. Regression can be graded as absent, partial, or complete; alternatively, a percentage scoring system has also been used and is advocated by the CAP.[12] Using the CAP approach, regression is categorised as involving more or less than 75% of the tumour volume. A third method is to measure the depth and width of fibrosis in millimetres.[23] Similarly, TILs can be stratified based on both their density and distribution pattern (focal or diffuse).[20] A standardised

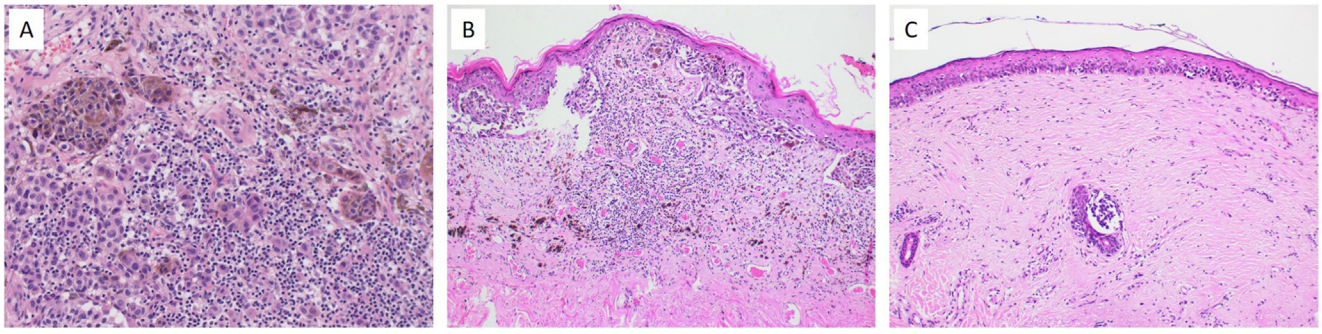

Fig. 1 Different stages of immunological host response in cutaneous melanoma. (A) Tumour-infiltrating lymphocytes are seen disrupting nests of melanoma cells at the bottom of the image. (B) Intermediate regression characterised by immature fibrosis, angiogenesis, chronic inflammation, and pigment incontinence. (C) Late regression characterised by mature paucicellular fibrosis and effacement of rete ridges.

methodology for the reporting of regression and TILs would facilitate uniformity across studies and the interpretability of prognostic data. The International Immuno-Oncology Biomarker Working Group (IOBWG) has proposed a standardised approach for assessing TILs in solid tumours including melanoma,[24] but it is yet to be validated.[25]

MECHANISTIC ASPECTS OF REGRESSION

It is generally acknowledged that regression in human cancers is primarily a consequence of activation of the immune system against the tumour cells.[26] However, the precise cellular mechanisms involved are vast and complex, and not completely elucidated. They include cells of the innate and adaptive immune system and their peptide products, as well as non-immune cells that can modulate the immune effects through interactions with tumour cells and release of cytokines. Furthermore, the immune system may exert an overall immunosuppressive effect, which is essential in healthy subjects for the purpose of self-tolerance. The ultimate outcome of tumour development depends on the complex interplay between different subsets of immune cells, whose precise phenotype determines their immunomodulatory properties. The complete set of local cellular and non-cellular components that influence the evolution of the disease is referred to as the tumour microenvironment (TME),[27] and its composition and functional interactions have been extensively studied in recent years.[28] A further complicating factor is that the interactions between tumour cells and components of the TME are not static; they can evolve and adapt during the time-course of the disease, thereby influencing its progression.[29]

Roles of immune and non-immune components of the TME

The T lymphocyte is recognised as one of the most important components of the immune response to melanoma. CD8+ cytotoxic T lymphocytes (CTLs) are the most important effector cells in the anti-tumour response.[30] T cell activation occurs through receptor-mediated recognition of tumour peptides presented to the T cell by a specialised antigen-presenting cell (APC). In the case of CD8+ T cells, this antigen presentation is achieved through the MHC Class I receptor expressed by the APC. The activated T cell is then able to destroy the tumour cell through direct lysis, in the presence of appropriate co-stimulatory signals. It can also secrete pro-inflammatory cytokines, such as IFN-γ, with diverse downstream effects.[31] However, this basic pathway of CD8+ T cell activation and tumour killing is complicated by a number of competing processes. It has been shown that chronic exposure to antigen alters the functional status of CD8+ cells such that they exhibit a decreased capacity for tumour lysis and an increased surface expression of inhibitory molecules, in a process termed 'T cell exhaustion'.[32]

Analogously, CD4+ T helper lymphocytes (T_h) are activated by binding antigen presented on MHC Class II molecules by APC. However, the CD4 surface marker is shared between several lineages of T_h cells, which differentiate via different transcription factors, secrete different sets of cytokines, and ultimately have distinct biological effects within the TME.[33] The T_h1 subtype is the best studied in melanoma, and is believed to exert anti-tumour activity. These cells exert their function via several mechanisms including cytolytic tumour cell killing and secretion of cytokines that promote cellular, humoural, or innate immune responses. On the other hand, another distinct subset of CD4+ cells is the regulatory T cell (T_{reg}), which expresses the transcription factor forkhead box protein 3 (FoxP3) and is known to suppress the immune response.[34] This is achieved in a number of ways including secretion of immunosuppressive cytokines, cytolytic killing of effector lymphocytes, high expression of co-inhibitory molecules, and sequestering of immune-activating cytokines.[35]

Dendritic cells (DC) are the most effective antigen-presenting cells and are necessary for proper activation of T lymphocytes. However, like other cells of the immune system, different subtypes of DC exert different functions and their precise immunomodulatory properties depend on their activation state. Thus, mature conventional DC (cDC) exert an anti-tumour effect by recognising antigen and presenting it to CD8+ T cells within lymph nodes, leading to T cell activation and tumour cell elimination. On the other hand, immature DC are incapable of activating T cells,[36] instead assisting tumour growth by promoting angiogenesis and Treg function.[37]

Macrophages can perform a wide range of functions depending on their type and influences within the TME. Some may act as antigen-presenting cells,[38] while others are phagocytic. Macrophages also release an array of cytokines and enzymes which can either promote inflammation or directly inhibit CTLs. Other tumourigenic mechanisms include expression of surface T cell inhibitory molecules[39] and promotion of angiogenesis.[40] Analogously to T_h cells, macrophage activation states can broadly be described as M1, which support immune activation against the tumour, and M2, which support tumorigenesis; however, this may be an oversimplification, as the actual spectrum of macrophage function may be more subtle and complex.[41]

Other immune cells in the TME include B lymphocytes, natural killer (NK) cells, and neutrophils. Their exact roles in anti-tumour responses are not well understood, and studies of prognosis often present conflicting results as various cell subsets and activation states are often not taken into account.[28] Briefly, B lymphocytes secrete antibodies and promote tumour killing through antibody-dependent cell cytotoxicity (ADCC); they may also act as antigen-presenting cells.[42] Studies in mice suggest B cells may be important for optimising T cell effector functions in melanoma.[43] There is increasing interest in B cells for their role in the formation of tertiary lymphoid structures, which may improve survival and treatment responses in melanoma.[44] NK cells are capable of direct cell lysis by secreting cytotoxic granules, and, along with neutrophils, may participate in ADCC. Tumour cells that have attempted to evade CTL attack are particularly vulnerable to NK-mediated killing.[45]

Apart from components of the immune system and melanoma cells themselves, the TME includes fibroblasts, the extracellular matrix (ECM), vascular and lymphatic channels, mesenchymal stem cells, and a number of other factors able to modulate the immune response to the tumour.[46] Fibroblasts are the most abundant mesenchymal cell type found in the peritumoural stroma. Along with other cells, they express matrix metalloproteinases (MMPs). These molecules are integral components of the ECM that, through proteolytic enzyme activity, are capable of remodelling the ECM. In melanoma, de-regulation of MMPs and their inhibitors, tissue

inhibitors of metalloproteinases (TIMPs), favours melanoma spread and invasiveness.[47] Studies have found differential expression of various TIMPs in melanoma lesions with and without regression, suggesting the existence of interactions between the immune system and ECM remodelling.[48]

Melanoma antigenicity and immune escape mechanisms

Immune activation is thought to be mediated through interactions between cells of the immune system and antigens expressed on the surface of tumour cells. Tumour antigens that are specifically recognised by lymphocytes fall into three categories: lineage-related or melanocyte differentiation antigens (MDAs), cancer/testis (CT) antigens, and oncogene-derived antigens.[49] MDAs are represented by normal non-mutated enzymes involved in melanin synthesis; these are present in both resident tissue melanocytes and melanomas and include tyrosinase, MART1, and gp100. CT antigens are highly expressed during development, but in adult tissues are found only in the placenta and testis. These antigens are aberrantly expressed by a variety of cancers, including melanoma. Finally, oncogene-derived antigens are generated by the numerous mutations within the tumour genome. The mutational burden of cutaneous melanoma is the highest of any malignancy,[50] a phenomenon partially responsible for the high antigenicity that characterises this tumour type.

One of the difficulties encountered by the immune system in its fight against the initiation and progression of melanoma is the high plasticity of the tumour, a property that allows it to modify its own cells and other cells within the TME in order to escape immune attack. One mechanism utilised by tumour cells is the reduced surface antigen expression, rendering the cell invisible to T cells.[51] Melanoma cells are also capable of releasing immunomodulatory factors into the TME,[52] which recruit T_{regs} and myeloid-derived suppressor cells, oppose maturation of dendritic cells,[9] and induce phenotype change in resident fibroblasts to a pro-tumour phenotype.[53] These altered melanoma-associated fibroblasts can secrete products that promote tumorigenesis and suppress immune functions.[54] Upregulation of co-inhibitory molecules on its cell surface allows the tumour cell to engage the corresponding receptors on CTLs, an interaction that induces anergy and CTL apoptosis.[55] In addition to these mechanisms, melanoma cells are able to deprive T cells of nutrients by sequestering them within their own cytoplasm.[56]

Spatial relationships between immune cells in regression

One important feature of the melanoma-immune system relationship is that the functional state of any given cell is highly dependent on its location and proximity to other components within the TME. In order to gain a deeper understanding of the immune mechanisms involved in regression, a number of investigators have attempted to characterise the immune infiltrate within regressive areas in melanomas. An early study noted that the distribution of CD8+ and CD4+ lymphocytes was highly heterogeneous between and within a set of primary melanomas. Furthermore, clonal expansion of CD8+ T cells, as assessed by PCR-based analysis, was not related to areas of histological regression or to the briskness of the TIL infiltrate.[57] This finding was against previous assertions that T cell clonality was a marker of an effective antitumour response.[58] Indeed, clonal expansion by itself does not reveal the functional state of the expanded clone, which may, for example, represent T_{regs}. In an immunohistochemical study,[59] Botella-Estrada and Kutzner compared the T cell subsets in regressing (halo) naevi to those in regressive melanomas. They found that markers of a cytotoxic immune response (TIA-1 and Granzyme B) were more frequent in halo naevi than regressing melanomas and hypothesised that immune clearance of melanoma cells may become defective in response to microenvironmental factors. Another study using immunohistochemistry compared the density of T_{reg} and S100A9+ cells in regressive vs non-regressive areas in primary melanoma.[60] In this study, S100A9+ was used as a surrogate marker for an immunosuppressive environment. The authors found that regressed areas contained fewer T_{regs} and S100A9+ cells than non-regressed areas, demonstrating a more robust anti-tumour response in areas of regression. In line with these findings, other studies of primary melanomas found reduced infiltration of anergic T cell subsets in areas of regression compared with both non-regressed areas in the same lesions and non-regressed control tumours.[61,62]

There are inherent challenges with interpreting immunohistochemical studies. First, regression is a dynamic phenomenon, so the immune cell composition in late regression may not reflect the stages preceding it. Second, from a technical standpoint, manual enumeration may not be sufficiently accurate or reproducible, and there is a lack of consensus on TIL scoring methods. Third, studying some T cell populations, such as CD4+ cells, is difficult due to overlapping expression of surface markers and their functional states being defined by differential expression of various cytokines.[33] Finally, the heterogeneity of study findings is likely to reflect the innate heterogeneity of melanoma cells themselves.

An alternative method for studying the functionality of lymphocyte subsets is by investigating various cytokine signatures by means of gene expression profiling. An early report showed that primary melanomas with evidence of regression had a higher expression of genes associated with the T_h1 phenotype compared with non-regressing tumours.[63] These findings were corroborated recently by Osella-Abate *et al.*,[64] who also demonstrated that expression of genes associated with macrophage activation was skewed towards the 'M1' phenotype in tumours with regression, compared with those without, which displayed a predominantly 'M2' signature.

Recent advances in microscopy and image processing allow for a high-resolution analysis of the TME with preservation of tissue architecture. Recently, Bosisio *et al.* have demonstrated that the functional state of TILs, as defined by the presence of co-stimulatory and co-inhibitory cell surface molecules, correlates significantly with the presence of regression.[65] In particular, lesions with late regression showed higher levels of immune activation compared with early or no regression. Furthermore, analysis of cell-to-cell interactions revealed that immune-stimulation interactions between different lymphocyte subsets were more prevalent in late regression; on the other hand, early regression represented a more complex network of cell interactions including those contributing to a defective immune response. The authors concluded that late regression reflects the biological endpoint of an effective TIL response, while a brisk

lymphocytic infiltrate does not necessarily equate to a true early phase of tumour clearance.

Prognostic implications of regression and TILs

Since the 1970s, the prognostic relevance of regression in primary melanoma has been the subject of controversy. At that time, it was already widely accepted that the depth of invasion was the strongest predictor of outcomes.[66] A number of authors sought to identify a subset of thin melanomas (≤0.76 mm) that harboured potential for metastasis, leading to the concept of so-called 'thin metastasising melanomas'.[67] These early studies found that thin melanomas with partial regression were associated with higher risk of metastasis and death than those without regression. One proposed mechanism was the enhancement of immune reaction against the tumour after lymph node seeding.[68] Other authors hypothesised that these tumours had possessed a greater depth of invasion and potential to metastasise before regressing, such that the measured Breslow thickness (BT) was an underestimation of its true value. To address this assumption, Traves *et al.* compared the depth of regressive changes with BT in 77 melanomas undergoing regression.[69] Whilst they found that the thickness of regression exceeded BT only for tumours ≤0.76 mm thick, that difference was insignificant and would not have resulted in upstaging of the tumours had the regression depth been considered the true BT. No survival analyses were conducted in this study due to low patient numbers. Other studies of prognosis showed no impact of regression on outcomes,[70–75] while still others demonstrated that regression had a protective role.[76–79] A meta-analysis that included 8557 patients found a lower risk of death when regression was present in the primary tumor.[80]

Regarding impact on lymph node status, a similarly wide range of findings is reported. A large meta-analysis of 14 studies and 10,098 patients concluded that regression in the primary tumour predicts a lower risk of a positive SLN,[81] a finding echoed by a number of other authors,[70,72,78,82,83] yet contradicted by others who found no correlation between regression and SLN status.[71,73] Still other studies reported an increased likelihood of spread to SLN in regressing melanomas,[84] with some specifically limiting their cohort to thin melanomas (<1 mm BT).[85,86]

These discrepant findings in the literature may be in part due to the inconsistent criteria used for defining regression as well as the subjectivity inherent to its histological assessment. The original definition of regression, proposed by Clark *et al.*, may be too restrictive as it requires a complete absence of tumour cells in the regressed area.[11] Furthermore, many studies did not include a definition of regression in their methods, while others excluded cases with >75% regression as these tumours were purported to exhibit distinct clinical behaviour.[61] Some studies established cut-off values below which regression was considered absent. Finally, there are inconsistent approaches for dealing with TILs, with some studies failing to control for this important feature, and others specifically excluding cases with TILs from analysis. Whilst TILs are generally regarded as a significant component of early regression, it must be recognised that the mere presence of TILs does not necessitate an effective anti-tumour response. Recently, Aivazian *et al.* conducted a prognostication study of 8693 patients, the largest single-institution study to date.[87] In this study, TILs were regarded as a separate parameter to established regression (defined by fibrosis, angiogenesis, and melanophages). The authors found that established regression and TILs were each independently associated with improved MSS and RFS, but importantly, the combination of both parameters predicted the best outcomes. These findings were corroborated by Tas *et al.*,[88] suggesting there may be a synergistic effect of established regressive fibrosis and an ongoing active anti-tumour immune response.

Despite incomplete understanding of the relationship between TILs and regression, prognostication studies of TILs have enjoyed better concordance in the literature. A large meta-analysis of 41 studies concluded that brisk TILs were associated with improved overall survival (OS), disease-specific survival (DSS), and recurrence-free survival (RFS).[89] Compellingly, a large analysis of population data including 2845 patients demonstrated increased protection from melanoma-related death with increase in TIL grade, when graded as absent, non-brisk, or brisk.[90] An Australian cohort of patients with tumours ≥0.75 mm thick also used a three-tier grading system and reported similar results.[91] More recent studies have similarly shown a correlation between TILs and improved RFS[92] and OS,[74,93,94] with many emphasising that a brisk infiltrate is required.[92–94] On the other hand, one study found no association between TILs and either OS or RFS;[75] however, the authors point out that they did not take into account the effect of systemic therapy in their analyses. Regarding sentinel node status, the literature reports consistently reduced risk of SLN positivity when TILs are present in the primary tumour,[74,87,91,92,95] with a minority of studies showing a lack of association.[75]

Prognostic impact of TIL subsets

Ultimately, studies of TILs and their impact on prognosis must be interpreted with caution due to the functional diversity of the infiltrate and the existence of tolerogenic immune states. In recognition of this fact, several authors have studied the various TIL subsets and other immune cells present within melanoma lesions and correlated them with patient outcomes. The heterogeneity of immune infiltrates is reflected in the conflicting results of survival studies examining the prognostic role of CD8+ T cells.[96,97] On the other hand, two markers of T cell activation, CD25 and OX40, were found to correlate with improved 5-year survival when found in the lymphoid infiltrate of primary melanomas.[98] Similarly, Granzyme B+ CTLs predicted a favourable clinical outcome in another study.[99] Whilst this result was not corroborated in a more recent investigation,[100] dual staining for Granzyme B and CD8 was not performed in that study, limiting the interpretability of these data. A recent study demonstrated that the functional status of the immune infiltrate, defined as 'active', 'transitional' or 'exhausted', was not only a good predictor of overall survival, but was a better prognostic indicator than the briskness of the infiltrate.[65] Concordant with this, Weiss *et al.* found that tumours with brisk TILs were associated with both improved clinical outcomes and enhanced expression of genes involved in T-cell activation pathways.[101] As might be expected, the presence of FoxP3+ T_{regs} in the infiltrate of primary melanomas was associated with a poor clinical outcome in a number of studies,[102,103] although a lack of association has also been reported.[101,104] In addition to identifying the presence of prognostically favourable immune subsets, spatial

analysis of the infiltrate can further increase prognostic power. Recently, Attrill *et al.* demonstrated that localisation of prognostically favourable CD8+ T cell subpopulations in close proximity to tumour cells was associated with reduced risk of melanoma recurrence.[105]

REGRESSION AND IMMUNOTHERAPY IN ADVANCED MELANOMA

Unravelling the mechanisms of anti-tumour immunity, combined with the observation that regression can also occur in melanoma metastases,[106] led to the development of novel immune therapies to fight advanced disease. A number of approaches have been investigated with variable efficacies and toxicity profiles; these include biological molecules such as cytokines,[107] vaccine-based approaches,[108] adoptive T cell therapy,[109] and immune checkpoint blockade. However, it is the last of these that has dramatically changed the outlook for patients with metastatic melanoma. Immune checkpoints are molecules and pathways that inhibit the immune response and are essential for preventing autoimmunity in healthy subjects. In melanoma, these pathways can be exploited by the tumour to escape immune attack; conversely, therapeutic blockade of these checkpoints can re-activate the immune system leading to augmented T cell responses and subsequent tumour rejection. T lymphocytes at the tumour site have been shown to express high levels of inhibitory checkpoint molecules PD-1 and CTLA-4, which impair their functions leading to an exhausted phenotype.[110] Ipilimumab, an agent that inhibits CTLA4 on T cells, was the first checkpoint inhibitor to improve survival in metastatic melanoma.[111] Subsequently, the anti-PD-1 inhibitors, pembrolizumab and nivolumab, demonstrated superior survival rates and lower toxicity compared with ipilimumab in phase III trials of patients with metastatic melanoma.[112,113] Combination therapy offers the highest survival benefit, albeit at the expense of increased toxicity.[113]

Despite the success of modern immunotherapy-based treatments, the prognosis of Stage IV melanoma remains poor, with as many as two-thirds of patients eventually suffering relapse.[114] The search for biomarkers of response continues to be one of the most active areas of investigation. To this end, deeper analyses of the nature, distribution, and cross-talk between melanoma and its TME are at the forefront of current research.[115]

It is not known whether spontaneous regression in the primary lesion can predict response to immunotherapy-based treatments. Nevertheless, clear links between the two phenomena have been demonstrated both clinically and immunologically. Vitiligo, a condition in which immune destruction of normal skin-resident melanocytes results in depigmented patches, has been reported in melanoma patients and is an immune-related adverse effect of checkpoint blockade.[116] Indeed, the presence of vitiligo-like depigmentation has been shown to be associated with better treatment outcomes.[117,118] The shared mechanism is an infiltrate of CD8+ T cells against melanocyte/melanoma-shared antigens, which serve as immune targets on both melanoma cells and normal melanocytes.[119] As mentioned earlier, regressed areas in primary melanoma samples have a decreased infiltrate of T_{reg} subsets compared with non-regressed areas; this same pattern had previously been observed in a case of vitiligo.[120] The shared immunological basis of spontaneous regression, vitiligo, and immunotherapy-driven tumour clearance raises the possibility that histological regression may serve as a predictive biomarker.[121] Furthermore, deeper understanding of the pathways involved in the immune regulation of these phenomena may lead to new therapeutic targets and enhanced treatment outcomes.[119]

CONCLUSIONS AND FUTURE DIRECTIONS

Tumour regression is an important histological parameter that should form part of the dataset in pathological reporting of primary cutaneous melanoma. However, a widely accepted standardised approach for its assessment is still lacking. Whilst currently regression does not influence clinical management of melanoma,[122] this may change as this parameter becomes integrated in future prognostication tools. Computational advances and emerging multiplexed single-cell approaches[123] can lead to new insights into the immune mechanisms underpinning melanoma regression. These novel technologies can provide spatial mapping, information about cell-cell interactions, and image numerous cell types simultaneously, thereby providing a more in-depth characterisation of the TME. Such insights may uncover novel targets for future systemic therapies. Finally, there is scope for analysing histological regression patterns and TIL subsets in tumour beds treated with neoadjuvant checkpoint blockade or targeted therapy.[124] Such studies may aid in understanding the mechanisms of drug response and resistance, as well as providing improved predictive biomarkers in systemically treated melanoma patients.

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