MINIREVIEW

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HYPE or HOPE: the prognostic value of infiltrating immune cells in cancer

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Interactions between immune and malignant cells have been known to have clinical relevance for decades. The potential for immune control is now being therapeutically enhanced with checkpoint inhibitors and other novel agents to improve outcomes in cancer. The importance of the immune infiltrate as a prognostic marker is increasingly relevant. In this minireview, we present an overview of the immune infiltrate and its spatial organisation, and summarise the prognostic value of immune cells in different cancer types. International collaborative efforts are standardising histopathologic reporting of the immune infiltrate, to allow application of these parameters in the clinical and research settings. In general terms, a 'pro-inflammatory' tumour microenvironment and infiltrating CD8-expressing T lymphocytes are associated with improved clinical outcomes in a broad range of tumour types. The inhibitory function of other immune cells, for example, myeloid-derived suppressor cells and regulatory T cells, appear to have a major role in disrupting the capacity for the immune control of cancers.

The immune system and malignant cells interact via a complex network. The importance of immune function in tumour development and control has been acknowledged for decades. As immunotherapy enters clinical practice, these underpinnings have more relevance as we try to identify predictive biomarkers for benefit from new therapies. The seminal papers describing the 'hallmarks of cancer' pronounced the capacity to avoid immune destruction as one of the requirements for malignancy (Hanahan and Weinberg, 2011). There is now a vast literature supporting immunosurveillance as a significant contributor to the natural history of malignancy. The interaction between tumours and the immune system has been described in three scenarios of 'immunoediting' (Schreiber *et al*, 2011); specifically: elimination (where immune surveillance successfully eradicates malignant cells); equilibrium (where the immune system exerts control over abnormal cells) and escape (where tumour cells evade immune mechanisms allowing growth and metastasis) (Mittal *et al*, 2014). In this mini-review, we aim to define the immune infiltrate and its spatial organisation as well as summarising the prognostic value of immune cells in different solid cancers.

SUBTYPES OF IMMUNE CELL INFILTRATE

Historically, studies have focused on the interaction between cytotoxic T lymphocytes and cancer cells. The role of other immune cells is also now recognised as contributing to the complex immune response in cancer, some of which promote tumour control and others facilitate cancer progression (Table 1; Figure 1).

LOCATION AND SPATIAL ORGANISATION OF THE IMMUNE CELL INFILTRATE

Although the distinction between peritumoural, stromal and intratumoural lymphocytes is made histopathologically (Table 2), this is likely an artificial segregation as this is a dynamic network that allows chemokine-generated cell movement between these areas.

A qualitative description of the interplay between tumour and immune cell infiltrate has been termed the 'immune contexture', and includes the location of specific immune cells, tertiary lymphoid structures (ectopic lymphoid aggregates that are generated during immune stimulation and exhibit structural characteristics of lymphoid organs), and the chemokines and cytokines involved in this microenvironmental organisation (Fridman *et al*, 2011). Methods for describing the immune infiltrate are a limitation of current pathological reporting. There remains a lack of consensus regarding reporting of tumour-infiltrating lymphocytes (TILs), including methods to subtype the infiltrating cells and their spatial organisation. International working groups are trying to create and validate reporting guidelines (Salgado *et al*, 2015). Immunophenotyping of the

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Table 1. Cell types in the tumour immune infiltrate		
	Expression	**Function**
Lymphoid		
Cytotoxic T lymphocyte	CD3, CD8	Recognise and lyse target cells through release of perforin and granzymes. Activated by dendritic cell antigen presentation via major histocompatibility complex (MHC) Class I antigen to T cell receptor. Apoptosis is induced in cells expressing specific antigen
Regulatory T lymphocyte (also called suppressor T cells)	CD3, CD4, CD25, FOXP3	Serve to maintain tolerant to self antigens; inhibit dendritic cell function of antigen presentation and thus inhibits both the expansion and the differentiation of T effector cells. Polyclonal Tregs appear to modulate differentiation and cell trafficking
T helper lymphocyte (Th cell)	CD4	Help to modulate immune responses. Activate and promote growth of cytotoxic T cells; maximise activity of phagocytes through interaction with MHC Class II; role in B cell antibody class switching (e.g., from immunoglobulin-M to immunoglobulin-G)
Natural killer cell (NK)	CD16, CD56	A subset of cytotoxic lymphocytes that can be activated in the absence of MHC Class I antigen presentation, thus an important component of the innate immune system
Myeloid		
Dendritic cells	CD40	Act to process and present antigen on MHC Class I, and via co-stimulatory molecules they serve to activate T lymphocytes
Myeloid-derived suppressor cells (MDSC)	CD11b, CD66b	Pathologically activated immature myeloid cells, with morphological and phenotypical similarity to mononuclear and polymorphonuclear cells. Prevent activation of T cells and have a role in promoting tumour growth and metastasis
Macrophages	CD68	Part of the innate immune defence with phagocytic capacity, and also have a role in adaptive immunity through activation of other immune cells via cytokine release. M1 macrophages are pro-inflammatory (largely driven by interferon (IFN)-gamma); M2 macrophages release anti-inflammatory cytokines such as IL4, IL10, TGF-beta and nuture tolerance

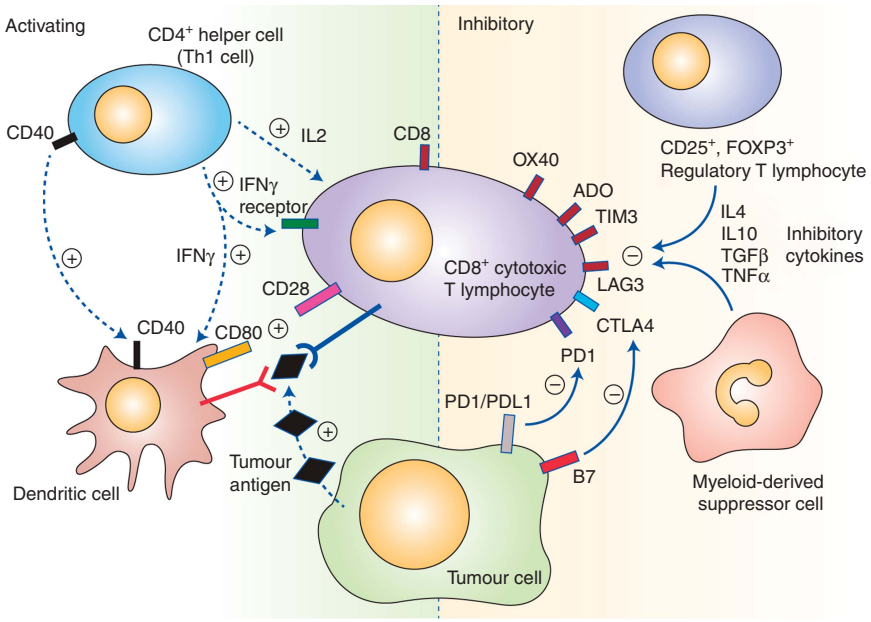

Figure 1. Pathways affecting cytotoxic T lymphocyte activity within the tumour microenvironment.

Table 2. Location of the immune infiltrate	
Infiltrate	**Location**
Intratumoural	Within the mass (or nest) of malignant cells, with direct proximity between cancer and immune cells
Stromal	In the surrounding connective tissues and blood vessels
Peritumoural	Around the tumour and can refer to cells at the advancing margin of the tumour, in the stroma or the tissues adjacent to the tumour

immune infiltrate by immunohistochemistry or immunofluorescence staining can be performed in tissue samples, or after generation of cell suspensions that are generated by mechanical or enzymatic breakdown of fresh tumour tissue (Stoll *et al*, 2015). The use of sectioned tissue specimens allows spatial understanding of cell position relative to tumour cells, however, similar to the use of cell suspension, is limited by challenges in antigen retrieval (capacity to bind identifying/specific proteins of interest) and poor standardisation. Novel approaches such as mRNA characterisation of immune co-regulated genes may help to identify and characterise the immune infiltrate (Stoll *et al*, 2015). Meta-analytical data suggest that in most cancers, the immune infiltrate is heterogeneous and there is limited reproducibility of leukocyte subtypes (Stoll *et al*, 2015). Although the presence of T cells is clearly important, the interplay between tumour antigens and major histocompatibility complex (MHC) molecules for antigen presentation is critical for efficient T cell activation. High affinity of the targeted peptides for MHC is required for strong stimulation of

T cells to secrete cytokines and produce tumour eradication or control (Engels *et al*, 2013). The specific antigenicity of coding exons in mutated cancer genes is an area of research and the capacity to sequence whole genomes with greater speed and reduced cost is enhancing the capacity to identify potentially antigenic mutations.

PROGNOSTIC VALUE OF INFILTRATING IMMUNE CELLS

The prognostic value of lymphocytes in stromal, peritumoural and intratumoural locations remains unclear, with conflicting data from different tumour sites. Peritumoural lymphocytes at the advancing tumour margin and those in direct contact with tumour cells have been purported to carry the most prognostic weight particularly in some disease sites (see below). In general terms, a 'pro-inflammatory' tumour microenvironment and infiltrating CD8-expressing T lymphocytes are associated with improved clinical outcomes in a broad range of tumour types. In contrast, the inhibitory function of other immune cells, for example, myeloid-derived suppressor cells and regulatory T cells (Tregs) appear to have a major role in disrupting the capacity for the immune control of cancers and are therefore associated with worse outcome.

Perhaps counter-intuitively, favourable outcomes have also been observed in tumours infiltrated by inhibitory immune cells, for example, forkhead box P3-positive regulatory T cells (FOXP3) cells in colorectal cancer. This may represent a feedback loop in the context of an existing anti-tumour immune response and thus actually indicate increased tumour immunogenicity (Gajewski *et al*, 2013). Myeloid-derived suppressor cells (MDSC) and tumour-associated macrophages are both capable of negative regulation of innate and adaptive immune pathways. MDSCs have a role in tumour growth and metastasis via promotion of immune privilege (ability to tolerate the introduction of antigens without eliciting an inflammatory immune response), tumour microenvironment remodelling, establishment of a pre-metastatic niche (a scenario where non-cancer cells promote future metastasis) and interaction with tumour to promote differentiation, invasion and angiogenesis (Marvel and Gabrilovich, 2015). There is evidence that MDSC expansion is associated with more advanced stages of malignancy in multiple cancer types and also correlates with poor prognosis independent of tumour burden (Ugel *et al*, 2015). Paradoxically, anti-tumour immunity also leads to selective pressure on malignant cells, which ultimately leads to survival of tumour cells with reduced immunogenicity (Shankaran, 2001). There are also data supporting the hypothesis that tumour-infiltrating immune cells can promote invasion and metastases (Man *et al*, 2013), which may in part explain the heterogeneity of results between studies examining this topic.

TUMOUR-SPECIFIC PROGNOSTIC VALUE

Breast cancer. In breast cancer, the presence of TILs is associated with improved prognosis in human epidermal growth factor receptor 2 (HER2) positive and triple negative breast cancers (TNBC), but not in luminal subtypes. In addition, the recognition of the prognostic value of the immune infiltrate has been the basis for establishing a breast cancer immunological grade (Salgado *et al*, 2015).

Independent of other clinicopathological prognostic factors or chemotherapy regimens, multiple studies have confirmed stromal TILs are associated with higher rates of pathological complete response (pCR) to neoadjuvant chemotherapy in all subgroups evaluated (including ER positive, HER2-positive tumours) (Dushyanthen *et al*, 2015). However, these differences in response only appear to translate into improved longer term outcomes in non-luminal tumours.

A meta-analysis of 25 published studies comprising over 22 000 patients, failed to show that immune infiltrates are associated with overall survival (OS) in unselected breast cancer patients, but did find such an association in TNBC (hazard ratio (HR): 0.79; 95% confidence interval (CI): 0.71–0.87). CD8-expressing lymphocytes were associated with improved disease-free survival (DFS; HR: 0.69; 95% CI: 0.56–0.84) and breast cancer-specific survival (HR: 0.78; 95% CI: 0.71–0.86) in the overall population, whereas the FOXP3-expressing lymphocytes were associated with worse DFS (HR: 1.47; 95% CI: 1.06–2.05) and OS (HR: 1.50; 95% CI: 1.15–1.97, $P = 0.004$) (Mao *et al*, 2016).

Clinical trials have not reported an association between TIL, nuclear grade or histopathological grade in TNBC with most making the assumption that TNBC are high grade (Adams *et al*, 2014). It remains uncertain whether this association may be explained partly by response to chemotherapy; lower grade luminal tumours have lesser response to cytotoxic therapy and are less frequently associated with infiltrating immune cells.

A Th1 immune phenotype and mRNA profiles consistent with immune activation have also been associated with response to neoadjuvant chemotherapy (Denkert *et al*, 2015). There is more variability in results seen in trials reporting outcome for CD4-expressing T lymphocytes and FOXP3-expressing Tregs. The presence of Tregs prior to chemotherapy is associated with higher probability of attaining a pathological complete response (pCR), which probably reflects their association with a higher number of CD8-expressing cells. A high ratio of CD8:FOXP3 cells and a lower proportion of FOXP3 at the end of neoadjuvant chemotherapy may have a more meaningful prognostic value (Dushyanthen *et al*, 2015).

The current working group have recommended semi-quantitative assessment of stromal TILs and at this stage do not advocate for sub-classification of lymphocytes (Salgado *et al*, 2015). This is due to both the greater reproducibility of stromal TIL measurement compared with intratumoural TILs, which are difficult to distinguish from malignant cells in standard H&E sections, and the fact that in TNBC and HER2-positive breast cancer, the prognostic power of TILs persists among all subtypes of infiltrating immune cells (Salgado *et al*, 2015).

Colorectal cancer. Several scoring systems have been proposed for quantifying the inflammatory response in colorectal cancer. These include the Jass score, the Immunoscore and the Klintrup–Mäkinen grade of overall peritumoural inflammation (Park *et al*, 2014). There is evidence that TILs are associated with greater prognostic value than the American Joint Committee on Cancer TNM stage (Jochems and Schlom, 2011). In a meta-analysis of nine trials examining tumour inflammation in colorectal cancer, the pooled HR confirmed an OS benefit for patients with prominent TILs compared with those without, with a HR of 0.59 (95% CI: 0.48–0.72, $P < 0.001$) and a HR for cancer-specific survival of 0.40 (95% CI: 0.27–0.61, $P < 0.001$). There were differences between all the studies in the thresholds used to determine TIL positivity of tumours, for example, some used mean or median cut offs, others used high *vs* low scores of Klintrup–Mäkinen or Jass scores (Mei *et al*, 2014). The evaluation of T cell subsets and specific location of lymphocytic infiltrate did not show strong prognostic value, specifically CD3, CD8, FOXP3 and at different sites (tumour centre, peritumoural stroma and invasive tumour margin) were examined. CD3-positive cells at the invasive margin had OR for DFS of 0.4 (95% CI: 0.35–0.68) and for OS of 0.63 (95% CI: 0.42–0.93). This analysis was limited by significant inter-study heterogeneity (Mei *et al*, 2014). This contrasts to earlier individual study data showing statistically significant association between the type of immune cell density at the centre of the tumour or the infiltrating margin and patient outcome (Jochems and Schlom, 2011).

Ovarian cancer. In a meta-analysis of 10 studies comprising 1815 patients with treated ovarian carcinoma (Hwang *et al*, 2012), presence of intra-epithelial T lymphocytes was associated with improved OS (pooled HR for death 0.45, 95% CI: 0.34–0.58, $P<0.001$). CD3- and CD8-expressing lymphocytes were both examined, and both conferred a survival advantage; CD8 was examined more frequently and demonstrated a larger magnitude of effect on OS than CD3 (pooled HR: 0.46 and 0.57, respectively) (Hwang *et al*, 2012).

This positive association between CD8-expressing lymphocytes and clinical outcome is also observed in the assessment of patients before treatment and following neoadjuvant chemotherapy. Data on CD3-expressing lymphocytes, B cells and NK cells are less clear (Santoiemma and Powell, 2015). There are conflicting data regarding FOXP3-positive Tregs, with a few studies demonstrating superior outcome, but most studies suggesting a negative impact on survival outcomes through inhibition of cytotoxic T cell activity (Santoiemma and Powell, 2015). The measured absolute number of infiltrating cells may not be as important as the proportion of CD8-expressing cells relative to all infiltrating cells. The prognostic value of intratumoural CD8-positive lymphocytes appears superior even to the adequacy of surgical debulking in prognosticating for both progression free survival and OS (Zhang, 2003).

Non-small cell lung cancer. In a meta-analysis of 29 trials with over 86 000 patients, high levels of CD8-expressing cells infiltrating the tumour or in the tumour stroma of non-small cell lung cancer (NSCLC) specimens were associated with better OS (HR: 0.76 and 0.80, respectively) compared with tumours without lymphocytes present. CD3 expression also demonstrated similar findings; pooled HR for OS 0.65 (95% CI: 0.50–0.84, $P=0.001$) for stromal CD3 cells and 0.66 (95% CI: 0.45–0.97, $P=0.03$) for intratumoural CD3 cells. Presence of intratumoural CD4-expressing cells between the tumour cells resulted in improved OS (HR: 0.65; 95% CI: 0.46–0.91, $P=0.01$). Despite a higher effect size, a significant association

Table 3. Studies examining the prognostic impact of infiltrating immune cells in melanoma

Study	Number	Result	Association	Cell type
(Kakavand *et al*, 2015)	60	Positive correlation between CD3, CD4 and CD8 cells in sentinel node and DFS/OS; PD1+ lymphocytes associated with worse outcome	Positive association	CD3, CD4 and CD8
(Saldanha *et al*, 2017)	655	Higher TILs are associated with better prognosis; confirms value of a simplified numerical TIL scoring system	Positive association	TIL
(Park and Kim, 2017)	177	Density of lymphocytes in the peritumoural and intratumoural regions were both prognostic	Positive association	TIL
(Obeid *et al*, 2016)	147	Expression of PD-L1 and PD-L2 correlated with increasing densities of immune cells. PD-L2 expression associated with improved OS	Positive association	PD-L2 and TILs
(Weiss *et al*, 2016)	1241	Melanomas with brisk TILs are defined by an immunostimulatory gene expression profile and improved prognosis compared with melanomas with non-brisk or absent TILs	Positive association	TILs
(Garg *et al*, 2016)	57	B cells are associated with a significantly better overall survival in patients with cutaneous primary melanomas of >1 mm Breslow depth	Positive association	B cells
(Bosisio *et al*, 2016)	710	Sheets/clusters of plasma cells associated with worse prognosis than melanomas without plasma cells	Negative association	Plasma cells
(Messaoudene *et al*, 2015)	39	NK cells in SLN associated with higher risk of relapse; NK cells did not correlate with thickness of primary but with patient age	Negative association	NK cells
(Fortes *et al*, 2015)	4133	High levels TILs associated with improved OS	Positive association	TIL
(Song *et al*, 2015)	82	TILs decreased the risk of distant metastases in oral mucosal melanoma	Positive association	TIL
(Donizy *et al*, 2015)	104	High levels TILs associated with improved OS	Positive association	TIL
(Eriksson *et al*, 2015)	4237	TILs demonstrated no prognostic value for survival	No association	TIL
(Thomas *et al*, 2013)	3330	High levels TILs associated with improved OS	Positive association	TIL
(Cintolo *et al*, 2013)	161	Absence of TIL was associated with worse DSS; In radial growth phase presence of TIL with regression was associated with a poor prognosis	Positive association	TIL
(Lee *et al*, 2013)	90	Brisk TILs were associated with improved prognosis in acral melanoma	Positive association	TIL
(Grotz *et al*, 2013)	250	TILs in elderly melanoma patients predicts both SLN metastasis and improved melanoma-specific outcomes	Positive association	TIL
(Azimi *et al*, 2012)	1865	TIL grade is an independent predictor of OS. Pronounced TIL infiltrate associated with excellent prognosis	Positive association	TIL
(Erdag *et al*, 2012)	147	Higher densities of CD8+ T cells correlated best with survival, a higher density of CD45+ leukocytes, T cells, and B cells also correlated with increased survival	Positive association	CD8, CD45, T cells and B cells
(Ladanyi *et al*, 2011)	106	CD20+ B cells most often found in peritumoural stroma, correlated with activated T lymphocytes and high number of these cells provided OS advantage	Positive association	CD20 B cells and activated T cells
(Knol *et al*, 2011)	102	High Foxp3 expression using qPCR predicts for worse progression free survival in stage III melanoma patients	Negative association	FOXP3
(Burton *et al*, 2011)	515	TIL response is a significant predictor of SLN metastasis but is not a major predictor of DFS or OS	No association	TIL

Abbreviations: DFS = disease-free survival; DSS = disease specific survival; NK = natural killer; OS = overall survival; SLN = sentinal lymph node; TIL = tumour-infiltrating lymphocytes.

Table 4. Immune cells in renal cell carcinoma

Study	Number patients	Result	Association	Cell type
(Geissler *et al*, 2015)	104	Tumour-infiltrating NK cells and Th1 markers associated with increased OS, for example, HLA-DRC and CXCR3C T cells; whereas a high number of T cells, especially with high CD69 expression correlated with worse prognosis	Positive association; negative association	NK and Th1; T cells (CD69)
(Kang *et al*, 2013)	199	PD1-positive or FoxP3-positive lymphocytes predicted poor OS survival	Negative association	FOXP3, PD1 + lymphocytes
(Hotta *et al*, 2015)	105	Low levels of memory T cells had improved OS	Negative association	Memory T cells
(Eckl *et al*, 2012)	41	NK cell percentage does not provide prognostic information	No association	NK cells
(Liotta *et al*, 2011)	30	Increase in both peripheral and intratumoural Tregs associated with worse prognosis	Negative association	Tregs
(Li *et al*, 2009)	125	Increased peritumoural Tregs are associated with worse prognosis in clear cell renal cell carcinoma	Negative association	Tregs
(Bromwich *et al*, 2003)	73	Increased CD4 + T cells associated with worse cancer-specific survival; no association demonstrated with CD8 + T cells	Negative association	CD4

Abbreviations: NK = natural killer; OS = overall survival.

Table 5. Studies of TILs in head and neck squamous cell carcinoma

Study	Number patients	Result	Association	Cell type
(Xu *et al*, 2017)	202	TIL level was an independent positive prognostic factor for DFS	Positive association	TIL
(Kogashiwa *et al*, 2017)	84	PD-L1 expression was associated with CD8 + tumour-infiltrating lymphocytes and better outcome in patients with locally advanced oropharyngeal SCC	Positive association	CD8
(Punt *et al*, 2016)	162	High number of T cells was correlated with improved DFS in HPV-positive oropharyngeal SCC; improved outcome correlated with active Th17 cells and lower IL-17(+) non-T cells	Positive association	T cells, Th17
(Nguyen *et al*, 2016)	278	Higher CD4 levels predicted improved OS and disease-specific survival	Positive association	CD4
(Caldeira *et al*, 2015)	28	Increased neutrophilic infiltration demonstrated in tumours with higher T stage; no correlation with survival	No association	Neutrophils
(Balermpas *et al*, 2016b)	161	CD8 + TILs constitute an independent prognostic marker in HNSCC patients treated with adjuvant chemoradiotherapy; prognostic benefit is apparent in HPV pos and neg subgroups	Positive association	CD8
(Partlova *et al*, 2015)	54	HPV-positive tumour showed significantly higher numbers of infiltrating IFNγ + CD8 + T lymphocytes, IL-17 + CD8 + T lymphocytes, myeloid dendritic cells and are associated with better outcome compared to HPV-negative	Positive association	IFNγ + CD8 + T cells, IL-17 CD8 + T cells
(Wolf *et al*, 2015)	39	CD68 + macrophages were found associated with positive nodes and poorer overall survival (not significant)	Negative association	Macrophages
(Ward *et al*, 2014)	270	TIL levels prognostic in HPV-positive HNSCC	Positive association	TIL
(Balermpas *et al*, 2014)	101	High infiltrating CD3 + and CD8 + cells correlate with survival outcomes with chemoradiation	Positive association	CD3 + and CD8 + T cells
(Nordfors *et al*, 2013)	203	Higher CD8(+) TIL counts correlated to a better 3-year OS in HPV pos; no correlation of CD4(+) TILs with survival outcomes	Positive association; no association	CD8; CD4
(Fraga *et al*, 2012)	70	CD57 + TILs do not correlate with survival outcomes	No association	CD57
(Wansom *et al*, 2012)	46	T-cell infiltration did not differ by HPV status; related to DSS and OS; after adjusting for HPV status, CD8, FoxP3, and total T cells were significantly associated with DSS and OS	Positive association	CD8, FOXP3 and total T cells
(Sun *et al*, 2012)	83	Tumour-infiltrating CD4 + CD25(high) Foxp3 + Tregs correlated with intratumoural COX-2 expression and were associated with a worse recurrence free survival in univariate but not multivariate analysis	No association	FOXP3
(Pretscher *et al*, 2009)	33	Intra-epithelial CD8 cells in metastatic lymph nodes and high CD20 + B cells in lymphoid tissue of lymph node metastases were associated with improved DFS	Positive association	CD8, B cells
(Badoual, 2006)	84	CD4 + CD69 + T cells are associated with improved OS	Positive association	CD4CD69 + T cells

Abbreviations: DFS = disease-free survival; DSS = disease specific survival; HNSCC = head and neck SCC; OS = overall survival; SCC = squamous cell carcinoma; TIL = tumour-infiltrating lymphocytes.

between stromal CD4-expressing cells and outcome was not observed (HR 0.43; 95% CI: 0.07–2.61, $P=0.36$), likely due to greater heterogeneity. FOXP3-expressing T cells in the tumour stroma had association with worse progression-free and OS (HR: 2.14; 95% CI: 1.68–2.72; $P<0.001$) and 2.67 (95% CI: 1.74–4.08; $P<0.001$, respectively) (Geng *et al*, 2015).

Melanoma. Checkpoint inhibitors were first approved in melanoma after a long history of interest in the immune response to these tumours after observation of spontaneous responses (Mihm and Mule, 2015). One histopathological definition of the immune response in melanoma categorised the immune infiltrating response as 'brisk', a scenario where lymphocytes are demonstrated in the entire tumour mass or along the advancing edge; 'non-brisk', where lymphocytes are seen focally in the centre of the tumour or along part of the invasive margin; or 'absent' with no tumoural lymphocytes at all or lymphocytes seen, but not interacting with melanoma cells. These subgroups provide prognostic information in historical studies. In one study, melanoma-specific death was 30 and 50% lower in the non-brisk and brisk groups, respectively, compared with the absent group (Mihm and Mule, 2015). In contrast, studies report no survival advantage with lymphocytic infiltrate particularly with respect to tumours of earlier stage and not in the radial growth phase (Ladanyi, 2015). However, overall, there is a large body of evidence documenting the prognostic value of the immune infiltrate in melanoma (see summary in Table 3).

Renal cell carcinoma. There is contradictory evidence regarding the role of the immune cell infiltrate in renal cell carcinoma. Multiple studies have demonstrated a worse outcome in patients with a neutrophilic, and/or lymphocytic infiltrate (Jochems and Schlom, 2011), a finding which appears reproducible (Table 4). The reasons for this are not clear.

Head and neck cancer. Several clinical trials have demonstrated that tumour infiltration by CD3- and CD8-expressing T cells correlates with improved disease outcome in chemoradiotherapy-treated patients with head and neck cancer. This positive prognosis holds true regardless of the human papilloma virus (HPV) DNA status (Balermpas *et al*, 2016a). Smoking-associated tumours with higher degrees of genomic instability and higher antigenicity would be expected to have increased potential to activate an immune response; however, this is not supported by clinical evidence. There is conflicting information regarding differences in the immune infiltrate in HPV-positive *vs* negative status (Wansom *et al*, 2012; Partlova *et al*, 2015); see Table 5.

Urothelial cancers. The approval of immunotherapy in the treatment of advanced urothelial malignancy suggests the relevance of the immune system. This is supported by most studies demonstrating the positive prognostic value of CD3, CD4 and CD8 T cells, and the negative association of FOXP3-positive T cells with survival, see Table 6.

Hepatocellular carcinoma. Several studies have examined the role of the intratumoural and peritumoural (parenchymal) infiltrate in hepatocellular carcinoma (HCC) (Table 7). High levels of FOXP3 Tregs are associated with worse DFS and OS. Two large meta-analyses performed in 2014 demonstrate the importance of FOXP3 in both the development and prognosis of HCC (Huang *et al*, 2014; Zhao *et al*, 2014). Gabrielson *et al*, 2016 applied the Galon Immunoscore (Galon *et al*, 2014) to HCC and confirmed its prognostic value, CD3 and CD8 cell densities predicted recurrence with ORs of 5.8 (95% CI: 1.6–21.8) and 3.9 (95% CI: 1.1–14.2), respectively. PDL1 staining was positively correlated with high CD3 and CD8 density and predicted a lower rate of recurrence (Gabrielson *et al*, 2016). The applicability of these tools remains limited by routine access to technology to subtype these T cells.

Other tumour types. The prognostic role of the immune infiltrate in less common malignancies is summarised in the Online Appendix.

BRAIN METASTASES

Although the central nervous system (CNS) has been purported to be an 'immune privileged' site, there is an increasing evidence supporting the role of immune infiltrating cells in brain tumours. In a study by Harter *et al*, TILs in brain metastases from different tumour types were quantified and associated with outcome. This was then validated in a breast cancer only brain metastases cohort. Carcinomas demonstrated more frequent stromal infiltration, whereas TILs in melanoma were more often diffusely infiltrative. High TILs level, high-programed cell death protein (PD)1 + /CD8 + and programed death ligand (PDL)-1 staining were associated with smaller tumours but there was no significant association with survival demonstrated (Harter *et al*, 2015). In contrast, Bienkowski and Preusser, 2015 provide a review of the literature in which they concluded that tumour-infiltrating lymphocyte density in CNS metastases were strongly associated with improved OS .

Table 6. Studies examining prognostic impact of immune cells in bladder cancer

Study	Number patients	Result	Association	Cell type
(Krpina *et al*, 2015)	115	CD3 + and CD8 + TIL are predictive of bladder cancer recurrence in patients with solitary low-grade non-muscle invasive bladder cancer	Positive association	CD3 and CD8
(Wang *et al*, 2015)	302	Intratumoural CD103(+) TILs inversely associated with tumour size. High CD103 + cells associated with improved OS.	Positive association	CD103 +
(Zhang *et al*, 2015)	131	Tumour-infiltrating CD4(+) T cell density emerged as an independent prognostic factor for OS (HR: 2.75; $P=0.004$)	Positive association	CD4
Knief *et al* (2016)	149	FOXP3/CD8 (OS: $P=0.013$, HR: 1.32, 95% CIs: 1.06–1.65) ratios were significantly associated with briefer OS and time to cancer-specific death	Negative association	FOXP3/CD8 ratio
(Sjodahl *et al*, 2014)	296	CD3(+) TILs was significantly associated with good prognosis. Positive association with CD3 was modulated by CD68(+) TAMs. Strongest negative association with survival was a high ratio between CD68 and CD3	Positive association; Negative association	CD3 + ; High CD68/CD3 ratio
(Sharma *et al*, 2007)	69	Higher numbers of CD8 TILs within the tumour (> or = 8) had better DFS and OS	Positive association	CD8
(Hilmy *et al*, 2006)	103	No correlation between TIL level and prognosis	No association	TILs

Abbreviations: TAM = tumour-associated macrophage; OS = overall survival; TIL = tumour-infiltrating lymphocytes.

Table 7. Studies examining prognostic value in HCC

Study	Number patients	Result	Association	Cell type
(Sideras *et al*, 2017)	154	Low CD8 + TIL associated with poor HCC-specific survival.	Positive association	CD8
(Gabrielson *et al*, 2016)	65	Intratumoural and peri-tumoural CD3 + /CD8 + density associated with lower risk of recurrence	Positive association	CD3/CD8 ratio
(Tu *et al*, 2016)	57	FOXP3 + Tregs/CD4 + T cells ratio was an independent prognostic factor for OS	Positive association	FOXP3/CD4 ratio
(Wang *et al*, 2016)	66	Tumour CD4 and CD8 lower than non-neoplastic liver; high Foxp3 associated with poor OS, whereas low CD8 expression in non-neoplastic liver associated with high HCC recurrence rate.	Negative association	FOXP3
(He *et al*, 2015)	149	High neutrophil to lymphocyte ratio in peritumoural tissues correlated with poor prognosis in patients with HCC	Negative association	Neutrophil:lymphocyte ratio
(Sun *et al*, 2015)	449	CD8 + in tumour centre had highest prognostic impact on DFS and OS	Positive association	CD8
(Ozgur *et al*, 2014)	8	High FoxP3 + poorer DFS	Negative association	FOXP3
(Brunner *et al*, 2015)	119	IL-33 and CD8 + cells associated with prolonged OS	Positive association	IL-33 and CD8
(Huang *et al*, 2014)	1964	OS significantly lower in high FOXP3 infiltrated tumours than low (at 1, 3 and 5 years) (meta-analysis 13 studies)	Positive association	FOXP3

Abbreviations: HCC = hepatocellular carcinoma; OS = overall survival; TIL = tumour-infiltrating lymphocytes.

Summary. Broadly speaking, the immune infiltrate can be classified as a 'pro-inflammatory' phenotype with infiltrating T cells and a cytokine profile consistent with immune activation. Immune control of tumours can occur spontaneously, and the presence of an immune infiltrate is generally a good prognostic sign. However, the immune infiltrate has variable effect in prognostic models depending on the tumour type, location of the cells and state of activation; the complexity of immune networks are likely oversimplified in current measurement models. Tumour evasion through inhibitory mechanisms may serve as a predictive marker for benefit from immunotherapy, which inhibits negative regulators of the immune system. Alternatively, the microenvironment may lack immune cell infiltration, and tumour resistance is likely through immune system ignorance (Gajewski *et al*, 2013), and therefore promoting immune activation is less likely to be successful in this setting.

For tumour-infiltrating immune cells to live up to the 'hype' of inducing and promoting long-term tumour control and contribute as valuable prognostic markers, their subtype (especially activated antigen specific cytotoxic T lymphocytes) and position (organised spatial response) need to be defined and measured in a standardised manner. Successful inclusion of immune cell markers in prognostic clinical models is becoming a realistic hope in some cancers.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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