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REVIEW ARTICLE OPEN

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Tumour-infiltrating lymphocytes: from prognosis to treatment selection

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Tumour-infiltrating lymphocytes (TILs) are considered crucial in anti-tumour immunity. Accordingly, the presence of TILs contains prognostic and predictive value. In 2011, we performed a systematic review and meta-analysis on the prognostic value of TILs across cancer types. Since then, the advent of immune checkpoint blockade (ICB) has renewed interest in the analysis of TILs. In this review, we first describe how our understanding of the prognostic value of TIL has changed over the last decade. New insights on novel TIL subsets are discussed and give a broader view on the prognostic effect of TILs in cancer. Apart from prognostic value, evidence on the predictive significance of TILs in the immune therapy era are discussed, as well as new techniques, such as machine learning that strive to incorporate these predictive capacities within clinical trials.

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INTRODUCTION

The interaction between cancer and immune cells in the tumour microenvironment (TME) is thought to be crucial for the control of the development and progression of malignant tumours. Accordingly, tumour-infiltrating lymphocytes (TILs) have been identified in primary tumour tissue, tumour-bearing lymph nodes and metastases of numerous cancer types. TILs are defined as lymphocytes within and around cancer cells and have been associated with a survival benefit [1–5]. However, the prognostic role of TILs still remains controversial within different types of tumours. In the 2011 issue of the British Journal of Cancer, we performed a systematic review and meta-analysis on the prognostic value of subtypes of TILs in a variety of solid cancer types, including ovarian, colorectal, lung, hepatocellular, and renal cell cancer [4]. In this review, the prognostic significance of intratumoral $CD3^+$, $CD8^+$, and $CD4^+$ T lymphocytes, and intratumoral $FoxP3^+$ T regulatory T lymphocytes (Tregs) was assessed. In the systematic review, Gooden et al. identified a positive association of intratumoral $CD3^+$ and $CD8^+$ TILs on overall and progression-free survival [4]. In contrast, intratumoral $CD4^+$ TILs were associated with a slightly improved overall survival (OS) and $FoxP3^+$ regulatory TILs were not associated with overall survival [4]. Over the past decade, TILs have not only continued to be proven of prognostic value but evidence is also starting to emerge of their predictive significance in the immunotherapy era [6, 7]. Hence, in this review, we discuss the follow-up work of our original publication in 2011. In addition, we will give insight into the prognostic value of T-lymphocyte subsets beyond $CD3^+$, $CD8^+$ and $CD4^+$ T lymphocytes and discuss how these data can be applied in clinical trials as well as a biomarker for immunotherapy.

Search strategy

The Pubmed database was searched without limits to identify all relevant studies to the subject. Diverse search terms were used, including, but not limited to "tumour-infiltrating lymphocytes", "TILs", "T cells", "T lymphocytes", "CD3 T cells, "CD8 T cells", "CD4 T cells", "FoxP3 T cells", "regulatory T cells", "B cells", "PD-1", "Programmed Cell-death 1", "CD103", "CD39", "TIGIT", "TIM-3", "LAG-3", "CTLA-4" "Prognosis", "Survival", "Cancer", "Outcome", "Tertiary Lymphoid Structures", "Machine-learning", "Deep-learning", "Immunohistochemistry", "Immunotherapy", "Checkpoint Inhibition", "Prediction", "Predictive Value", "Biomarker".

When available, studies were included starting from 2012, when not available older literature was used.

Prognostic value of 'classical' TILs. In 2011, we reported on a systematic review and meta-analysis of survival associated with the presence of $CD3^+$ tumour-infiltrating lymphocytes (TILs). Briefly summarised, $CD3^+$ TIL infiltration was associated with both progression-free survival (PFS) (HR 0.53; 95% CI 0.39–0.73) and overall survival (OS) (HR 0.58; 95% CI 0.43–0.78) across malignancies [4]. Since publication in 2011, the advent of high-dimensional cytometric, mass spectrometry, and RNA-based single-cell analysis of $CD3^+$ TILs has significantly improved our understanding of this heterogenous T cell population, discussed in more detail below. Nevertheless, as a single marker for the prognostic benefit of T cells in tumours, high $CD3^+$ TIL infiltration has proven highly reliable in follow-up studies and has been associated with an improved OS in almost all cancer types, including melanoma and breast, colorectal, gastric, hepatocellular,

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and head and neck cancer [8–15].

Apart from the frequently used parameters of presence and density of TILs, recent studies also show that the location of TILs and their spatial organization may carry additional prognostic value. Accordingly, a meta-analysis of Mei et al. showed that a high $CD3^+$ TIL infiltrate in the invasive tumour margin had a positive correlation with OS (HR 0.63; 95% CI, 0.42–0.93) and disease-free survival (DFS) (HR 0.63; 95% CI, 0.35–0.68) in patients with colorectal cancer [11]. Conversely, a high $CD3^+$ TIL infiltrate in the tumour centre or tumour stroma had no association with OS or DFS. Elomaa et al. recently made similar observations and found that high proximity and density scores of T lymphocytes were associated with better cancer-specific (CSS) and OS and they suggest that there are stronger survival associations based on the invasive margins as compared to the tumour centre [16]. Thus, CD3 has remained a reliable and consistent marker for the identification of tumour-infiltrating T cells with prognostic benefit across cancer types.

In addition to $CD3^+$ T cells, our original work also made a crude distinction between the prognostic value of different T cell subsets. Perhaps the most obvious, although arguably least exciting, division in T cell subsets at the time was the classic differentiation of cytotoxic CD8 vs helper CD4 T cells. As described by Gooden and others, the presence of $CD8^+$ TILs is associated with prognostic benefits for all tested survival endpoints (OS, DSS, PFS) [4] and across solid cancers. For CD8 in particular, large cohort studies have demonstrated robust prognostic benefit. For instance, a meta-analysis of 14 studies with 2015 patients in hepatocellular carcinoma found a positive correlation between high levels of $CD8^+$ T lymphocytes and OS (HR 0.71; 95% CI 0.51–0.99; $P = 0.04$) and DFS (HR 0.66; 95% CI 0.47–0.92; $P = 0.01$) [17]. In ovarian cancer, a large cohort study of 1815 patients demonstrated that the presence of epithelial $CD8^+$ T lymphocytes was concomitant with improved OS (HR 0.45; 95% CI 0.34–0.58; $P = 0.001$) and PFS (HR 0.46; 95% CI 0.25–0.67), independent of clinicopathological variables. In most studies, the presence of $CD8^+$ TILs had a larger magnitude of effect than the presence of $CD3^+$ TILs [18, 19]. However, even though $CD8^+$ TILs are associated with prognostic benefits, the diverse functional profiles and markers of $CD8^+$ TILs in the TME makes the interpretation complex [19]. Maibach et al. have proposed to label activation markers and/or effector molecules when it comes to the prognostic value of $CD8^+$ TILs [3], a suggestion in-line with functional studies discriminating bystander from cancer cell-reactive $CD8^+$ T cells.

In contrast to the above-described $CD8^+$ counterparts, $CD4^+$ TILs have received less overall attention in the field of tumour immunology. However, recent work on e.g., follicular helper cells and class I-negative tumours are steadily shifting this paradigm [20, 21]. In part, the difference in contribution may stem from the complexity in $CD4^+$ T-cell differentiation. $CD4^+$ T cells can broadly differentiate into immune-promoting T helper (Th) subsets, as well as the highly immune suppressive $CD4^+$ regulatory cells (Tregs) with counteracting roles in anti-tumour immunity. Although their functions differ, we originally concluded that high $CD4^+$ TILs were associated with a slightly improved OS in oesophageal, gastric, hepatocellular, renal cell and ovarian carcinoma. However, we did not show an association between high $CD4^+$ TILs PFS and disease-specific survival (DSS). Yet, data on the prognostic value of $CD4^+$ TILs in more recent meta-analysis is conflicting. In hepatocellular, gastric, and colorectal carcinoma, no significant correlation was found between high $CD4^+$ TILs and OS [15, 17, 22]. Nonetheless, in most cancers, including melanoma and head and neck, ovarian, cervical, and cholangiocarcinoma, a positive association was reported between high $CD4^+$ TILs and OS and/or PFS [8, 13, 14, 23–27]. As these results did not all distinguish different CD4 T cell subsets, it remains at present impossible to determine whether the prognostic value of $CD4^+$ TILs is dependent on cancer type or heterogeneity in CD4 subsets, or both. Nevertheless, in studies where a (partial) discrimination was made, the $CD4^+$ subtype, Th1, seemed to be a more consistent prognostic factor. For example, in breast cancer and primary melanoma, the expression of Th1-associated genes predicted an improved OS [3, 23]. Unfortunately, no recent meta-analyses reported the effect of $CD4^+$ TILs on DSS in solid cancers.

The same conflicting reports on survival exist for $FoxP3^+$ CD4 Tregs. In 2011, we concluded that Tregs did not have an impact on OS, DSS and PFS in most solid cancer, including ovarian, endometrial, cervix, breast, hepatocellular, renal cell, gastric and colorectal carcinoma. Studies from 2012 until 2022 report great discrepancy in the prognostic value of $FoxP3^+$ TILs within variable tumour types [9, 11, 14, 15, 17, 28–36]. Contrary to the findings of Gooden et al., $FoxP3^+$ TIL infiltration has been associated with poor prognosis for OS and relapse-free survival (RFS) in most cancers, such as breast, hepatocellular, gastric, ovarian, cervix, and cholangiocarcinoma [17, 26, 28, 29, 32, 35–37]. However, Asahi et al. [34] found no statistically significant correlation between high density intratumorally $FoxP3^+$ TILs and OS in gastric cancer and cholangiocarcinoma. In addition, in melanoma $FoxP3^+$ TILs were not a negative nor positive prognostic factor. Interestingly, in colorectal and head and neck cancer high levels of intratumorally $FoxP3^+$ were associated with a good prognosis [14, 28, 29, 31]. Based on these diverse findings, biological properties within different microenvironments of specific tumour (sub)types seemed to play an important role. In fact, an hypoxic and acidic TME has been associated with the upregulation of chemokines that enhance recruitment of Tregs to the tumour and increase activity of these Tregs in the tumour, which highlights the importance of the TME [38–42]. Furthermore, Saito et al. [30] proposed that the discrepancies in prognostic value of $FoxP3^+$ TILs could be attributed to different subtypes of $FoxP3^+$ TILs, namely suppression-competent $FoxP3^{hi}$ Tregs and non-suppressive $FoxP3^{low}$ Tregs. Hence, attention is shifting towards analyses of subtypes of $CD8^+$, $CD4^+$ and $FoxP3^+$ TILs instead of the population as a whole.

Finally, an analysis strategy that may generate more predictive data for survival than scoring individual TILs is to determine the ratios between T lymphocyte subsets. At the time of our original work, there were limited studies incorporating T lymphocyte ratios. As a consequence, we were only able to perform a pooled analysis on the $CD8^+/FoxP3^+$ ratio and concluded that a high $CD8^+/FoxP3^+$ ratio has a strong positive relation with OS. In the last decade, this association was validated in different studies in which a positive relation between a high $CD8^+/FoxP3^+$ ratio and better survival outcomes was observed [17, 43]. In addition, several studies have reported on the association between $CD4^+/FoxP3^+$ ratio and OS. Like the $CD8^+/FoxP3^+$ ratio, high $CD4^+/FoxP3^+$ ratios correlated with improved OS [17, 27, 33, 44].

Prognostic value of 'novel' TIL subsets. In 2011, our review primarily focused on the prognostic value of the classic subsets of TILs which was limited to $CD3^+$, $CD4^+$, $CD8^+$ and ratios between these subtypes. However, in the last decade, substantial developments have taken place with respect to the identification and prognostic value of TILs beyond these classic T cell subsets, including subsets defined by markers CD103, CD39 and PD-1.

CD103, also known as integrin αEβ7 (*ITGAE*), is a heterodimeric transmembrane protein expressed primarily on epithelial-associated lymphocytes that is involved in cell adhesion, migration and lymphocyte homing of cells through binding to E-cadherin [45, 46]. Since E-cadherin is expressed in epithelial cells, CD103 TILs have been associated with immunity against cancers of epithelial origin. As a result, $CD8^+CD103^+$ TILS were strongly associated with increased OS, DSS and/or RFS in most cancer types, including urothelial cell carcinoma and ovarian, cervical,

endometrial, breast, colorectal, gastric, and head and neck cancer [13, 47–61]. Importantly, the favourable prognostic value of CD103 was only found for intratumoral $CD103^+$ TILs and for $CD8^+$ TILs expressing $CD103^+$. In fact, stromal $CD103^+$ TILs were not associated with prolonged DFS and OS, and $CD4^+CD103^+$ TILs were associated with poor OS in gastric cancer [61, 62]. Contrary to other cancer types, $CD8^+CD103^+$ TIL frequencies in cutaneous squamous cell carcinoma were significantly associated with the development of metastasis and worse prognosis [63]. The same was found in clear cell renal cell carcinoma (ccRCC) in which high density of $CD8^+CD103^+$ predicted worse OS [64]. Interestingly, Duhen et al. [65] investigated the prognostic role of co-expression of CD39 and CD103 on $CD8^+$ TILs and concluded that dual expression of both CD103 and CD39 was consistently better at predicting survival than CD103 alone. Altogether, these data show that $CD103^+$ TILs have a positive prognostic value in most solid cancers and that co-expression of CD39 may improve the prognostic value of CD103 alone.

CD39, encoded by the gene *ENTPD1*, is an ectoenzyme that is responsible, together with CD73, for the generation of an immunosuppressive form of adenosine by converting adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and cyclic adenosine monophosphate (cAMP) [66]. In the context of cancer, only limited and conflicting literature is available on the prognostic value of $CD39^+$ TILs. In hepatocellular carcinoma (HCC) and rectal adenoma carcinoma, a higher frequency of $CD8^+CD39^+$ TILs was positively associated with improved OS [67, 68]. In contrast, CD39 expression was significantly associated with advanced tumour stage and worse survival rate in ccRCC, and bladder and small cell lung cancer [69–71]. Interestingly, in lung cancer and ccRCC, high expression of CD39 was correlated with abundance of immune suppressive factors, such as $FOXP3^+$ and $PD\text{-}1^+$ TILs [69, 72]. In fact, ccRCC patients who received immune checkpoint blockade (ICB) with high CD39 expression exhibited favourable OS compared to ccRCC patients with low CD39 expression [69].

Finally, the TIL marker that has received arguably the most attention in recent years is Programmed cell death protein 1 (PD-1). PD-1 is an inhibitor of both adaptive and innate immune responses and a marker of exhaustion in TILs displayed on the surface of both activated $CD4^+$ and $CD8^+$ T lymphocytes [73]. While several studies report on the prognostic effect of PD-1-ligand 1 (PD-L1), the prognostic effect of PD-1 expression on TILs is less frequently examined. Nevertheless, studies reporting on this topic present a varying view on the matter. Studies performed in patients with intrahepatic cholangiocarcinoma and nasopharyngeal carcinoma reported lower overall survival and a higher recurrence rate [27, 74], while studies performed in patients with HGSOC and NSCLC reported a positive correlation between the presence of $PD\text{-}1^+$ TILs and disease-specific survival and OS respectively [75, 76]. This might suggest that the prognostic effect of PD-1 depends not only on its presence but also on tumour type. Interestingly, recent research by Thommen et al. looked into a transcriptionally and functionally distinct $PD\text{-}1^+$ $CD8^+$ T cell pool. They reported that high expression of PD-1 is also associated with overexpression of other inhibitory receptors such as TIM-3, LAG-3, TIGIT, 2B4 (CD244) and BTLA, where the first two were almost exclusively found on TILs with high PD-1 expression [77]. Even though upregulation of PD-1 has a detrimental effect on classic $CD8^+$ T cell functions such as cytotoxic activity and cytokine production and secretion, the study shows that TILs with high expression of PD-1 also acquire novel effector functions. Specifically, the production and secretion of the effector chemokine *CXCL13*. As a single-receptor chemokine, CXCL13 binds only to CXCR5, which is expressed on B cells and certain types of $CD4^+$ T cells. This suggests that the secretion of CXCL13 by $PD\text{-}1^+$ TILs attracts B cells and CD4 cells to the TME. This is substantiated by the fact that most of the $CD8^+$ T cells with high PD-1 expression are colocalized with—among others—B cells in tertiary lymphoid structures (TLS). This new acquired effector function of exhausted PD-1 expressing T cells make them a possible predictor for response to PD-1 targeting therapies [77, 78]. Finally, although not studied extensively in meta-analyses, the presence of exhaustion markers TIGIT, TIM-3, LAG-3, CTLA-4 alone have been alternately correlated with OS and recurrence rate in isolated studies [79–83].

The prognostic value of TIL heterogeneity. Most studies evaluating the prognostic value of 'classical' and 'novel' TILs in solid tumours have been limited to the assessment of individual TIL markers by immunohistochemistry or mRNA expression. As density-based analyses of TILs are performed on biopsy slides that capture only a small part of the tumour, these assessments do not entirely explore the characteristics (and therefore prognosis) of tumours, including the spatial heterogeneity of TILs in the TME [84, 85]. In addition, immunohistochemistry on H&E slides is interpreted by pathologists, resulting in a highly subjective prognostic tool with a restricted reproducibility [86]. Recently, new technological breakthroughs in pathology and radiochemistry, such as machine learning and immune-positron emission tomography (immune-PET), have been developed that hold great promise in refining the prognostic value of TILs.

In 2012, Krizhevsky et al. [87] introduced convolutional neural networks, followed by the appearance of machine-learning (ML) models in pathology. In immune-oncology, ML, and in particularly deep learning (DL), has proven an unbiased and reproducible tool to identify histopathological patterns based on fully automated computer-aided image analysis of routinely generated H&E-stained slides. This is done by a.o. assessing consistency in the expression of immunohistochemical markers, tumour morphology, molecular alterations, and spatial distribution of TILs and cancer cells [85]. ML models have already been trained in a variety of cancers [84, 88–97].

Image-based DL algorithm to quantify TIL densities and to assess spatial heterogeneity of TILs might therefore augment the prognostic value of TILs significantly [88–90]. Heindl et al. [88] developed an image-based DL tool to score lymphocytic infiltration based on spatial heterogeneity of TILs in breast cancers and concluded that these scores were highly prognostic, particularly for late recurrences. Moreover, Horeweg et al. [89] confirmed that the integration of image-based quantification of intraepithelial $CD8^+$ cells superseded the prognostic utility of the standard molecular endometrial cancer classification in early-stage endometrial cancer. Accordingly, prognostic image-based DL models have the advantage that they can potentially take into account the spatial interaction among TILs and cancer cells which has proven to have a predictive value in tumour progression and recurrence.

Altogether, these studies show that the prognostic value of TILs in clinical practice could be further enhanced by the utilisation of ML models.

TILs as a biomarker for immunotherapy. Blocking the above-mentioned PD-1-PD-L1 axis with monoclonal antibodies (MAbs) has increased therapeutic options in solid tumours. However, a significant group of patients do not benefit from PD-1/PD-L1 blockade, but are exposed to (the risk of) treatment-related toxicity. Because of this, there is a clinical need for prognostic and predictive biomarkers that can help reduce possible overtreatment. A logical biomarker of interest are TILs. A recently performed systematic review by Presti et al. gives a thorough overview of current research on TILs as a predictive biomarker [98]. A high baseline TIL density is associated with improved outcome (ORR in metastatic and pathological complete responses (pCR) in early disease) in several solid tumours treated with immune checkpoint inhibitors including melanoma, breast cancer,

endometrial cancer, CRC, and NSCLC [7, 28, 99–111]. Furthermore, trials that evaluated on-treatment histological samples showed that the dynamics in TIL density during treatment was associated with improved outcome, even when there was no association with baseline TILs observed [100, 112]. Next to the density and variation of TILs in the TME, spatial distribution also influences the response to ICB. The ratio between the invasive margin and the tumour centre might be of special interest as it has been shown to provide additional information on early changes after administration of ICB in and around the tumour and might be an early predictive biomarker for treatment effects [101, 102, 107, 113–115]. Findings like these show that the TME is a dynamic system that constantly levitates on changes in the tumour-host interaction and that the dynamics in TILs, especially during and after treatment with ICB, can be of prognostic and predictive value in patients treated with ICB.

The importance of the TME on TIL dynamics is further proven by the effect of hypoxia and acidity on TIL effector functions and proliferation. It has been suggested that an acidic environment prevents lymphocyte proliferation by impairing the stimulatory activity of IL-2 [76, 116, 117]. Furthermore, acidosis in the TME impairs the cytolytic activity and cytokine secretion of $CD8^+$ T lymphocytes [76, 118]. Hypoxia, often due to chaotic and insufficient tumour microcirculation [119], has detrimental effects on effector functions of both $CD4^+$ and $CD8^+$ T lymphocytes and supports the proliferation and migration of immunosuppressive cells. Furthermore, Zandberg et al. showed that the effect of anti-PD-1 therapy is diminished with increased hypoxia in HNSCC [120].

Apart from focusing on the predictive and productive value of TILs as a whole, alternative biomarkers such as TIL subsets (e.g., $CD4^+$, Tregs, T-memory, $CD8^+$), the expression of exhaustion (e.g., PD-1, TIM-3, LAG-3) and activation (e.g., granzyme B) markers and their association with clinical outcome after ICB treatment [108, 109] have also been studied. Even though these studies do not show a definitive function of one of these markers as a predictive biomarker, it shows the interaction between TILs and the TME in response to ICB treatment. Furthermore, the predictive value of a certain TIL can vary depending on the type of ICB that is used [109]. Even though these data on TILs in tumours show a possible predictive role of TILs in association with ICB treatment, a large number of studies lack an ICB-free arm for comparison complicating definitive conclusions. Finally, a high infiltration of TILs and the presence or absence of exhaustion and/or activation markers is not always correlated to a good response or any response at all. An example of this is ovarian cancer where several clinical trials with immune therapy show little-to-no response to ICB, even in tumours with high densities of TILs [121, 122].

DL may also play a crucial role in the selection of patients for immunotherapy. Indeed, Saltz et al. [84] used image-based DL to cluster spatially connected regions of TILs and found differences in cluster dispersion between melanoma, a cancer type that is highly response to immunotherapy, and breast cancer, a cancer type that is generally unresponsive to immunotherapy. Likewise, Chen et al. [91] identified and validated three distinct immune subtypes presented with diverse components of tumour-infiltrating immune cells, molecular features, and clinical characteristics in gastric cancer by using unsupervised consensus clustering algorithm. Thus, each cancer subtype may benefit from different immunotherapy strategies. Next to image-based DL, immune methylome signatures queried by ML were also shown to be predictive for immunotherapy response. Duruisseaux et al. found a correlation between epigenetic features based on DNA methylation signature (EPIMMUNE) and clinical benefit with PD-1 blockade in NSCLC. NSCLC tumours of non-responders to immunotherapy were enriched with cell populations derived from the myeloid lineage, while responders were enriched with cell populations derived from the lymphoid lineage [92]. Based on these studies (mixed), ML models may be a valuable tool to select patients for immunotherapy.

Image-based (TIL) DL has also gained attention for predicting the status of molecular pathways, including microsatellite instability (MSI) and mismatch repair deficiency (MMRd) status. In colorectal cancer, variable image-based DL models have been designed that exceeded the performance of experienced gastrointestinal pathologist at predicting MSI on H&E-stained slides [93–95]. Within these models, the presence of TILs and their spatial orientation in the tumour had important predictive value [94–97]. For instance, Lee et al. [96] confirmed that their image-based DL-model discriminated MSI-high and microsatellite stability (MSS) largely based on high TIL and peritumoral lymphocytosis. In addition, Schrammen et al. [94], Bilal et al. [95] and Kather et al. [97] found that lymphocyte-rich tumour regions, high proportions of TILs and necrotic tumour cells were most predictive for MSI in their image-based DL models. Since, MSI/MMRd status of a patient has therapeutic consequence, a cost-efficient image-based DL model using, e.g., TILs may prove to be an efficient tool to triage patients for confirmatory MSI/MMRd testing.

Perspectives. It is evident that TILs are associated with improved long-term survival across malignancies. A major challenge is to now translate these associations into clinically relevant or clinically actionable information. Indeed, while TIL 'scores' may help supplement molecular information and improve patient counselling on the likelihood of recurrence, validated systems that can be implemented into routine clinical practice are scarce. This is true for both standardised scoring systems for pathologists, but also machine-learning algorithms. Arguably the most substantial obstacle that has hampered this clinical translation is the heterogeneity in spatiotemporal distribution of immune cells, and the underlying intra-immune cell heterogeneity. These problems are compounded by the apparent incongruity between the prognostic value of TILs in malignancies, and the potential likelihood of response of a malignancy to TIL-targeted immunotherapy, such as immune checkpoint inhibitors. A prototypical example of this paradox, ovarian cancer, has long been known to harbour tumour-reactive TILs with prognostic value, but only marginal responses to immune checkpoint inhibitors have been observed so far. Furthermore, as the prognostic value of TIL infiltrate in ovarian cancer appears to be restricted to a subgroup of primary patients with complete cytoreduction, it will be interesting to determine whether this subset also responds to ex vivo immune checkpoint inhibitor treatment in e.g., patient-derived tissue fragment (PDTF) models, or whether the prognostic and predictive value of TILs are uncoupled in these patients. As recent work points to tissue-resident memory-driven immune responses in ovarian cancer, and the therapeutic benefit of ICB is more and more linked to the involvement of (secondary) lymphoid organs, a more complex view on prognostic tissue-resident and predictive systemic immune responses may develop in the coming years.

An exciting development herein, both from patient and translational perspective, is the advent of neo-adjuvant ICB. Most successfully applied in MMRd CRC, neo-adjuvant ICB is associated with remarkable rates of pathologic complete responses. While a general trend is observed in these studies for higher levels of baseline $CD8^+$ TILs in responding patients, the same trend does not hold for $CD3^+$ TILs, and responses on the individual patient level are more heterogenous. With studies using radiolabeled immune imaging agents (e.g., CD8 and PD-1) now underway to tackle the problem of sampling heterogeneity, it will be interesting to see these modalities applied within the neo-adjuvant setting. Ideally, these whole-body immune monitoring agents will be applied in combination with an in-depth assessment of TLS and tumour-draining lymph nodes (TDLNs). Both TLS and TDLNs have been proposed as reservoirs for precursor-

exhausted T cells that maintain long-term immunity against chronic stimulation by cancer cells, and have been linked to response to ICB, in many instances with superior predictive power to TIL status. However, major outstanding questions on the relationship between TILs, TLS and TDLNs remain to be addressed, most notably whether T-/B cell clones are shared across these sites, whether unique phenotypes exist across these sites, and how these cells dynamically respond to treatment with ICB. Technological developments on machine learning combined with high-dimensional techniques such as imaging mass cytometry and spatial transcriptomics are now starting to shed light on these questions, and it will be exciting to see this field develop over the coming years.

Overall, the analysis of the prognostic value of TILs combined with the clinical success of ICB-therapy has sparked an amazing development in our understanding of local and systemic tumour/immune cell interactions. Ever advancing high-dimensional assessment of immune cell control of tumours will need to be paired with an effective practical translation of this information into understandable, relevant and actionable information for use in clinical practice.

DATA AVAILABILITY

Not applicable.

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KB and ALE drafted and revised the manuscript. MB and HWN conceived the project, supervised writing and revised the manuscript. All authors read and approved the final version.

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Authors HWN and MdB hold a patent on antibodies targeting CD103 (no 62/704,258).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT TO PUBLISH

All authors have approved to publish this manuscript.

ADDITIONAL INFORMATION

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