The clinical role of the TME in solid cancer
Nicolas A Giraldo, Rafael Sanchez-Salas, J David Peske, Yann A Vano, Etienne Becht, Florent Petitprez, Pierre Validire, Alexandre Ingels, Xavier Cathelineau, Wolf Herman Fridman, et al.

To cite this version:
Nicolas A Giraldo, Rafael Sanchez-Salas, J David Peske, Yann A Vano, Etienne Becht, et al.. The clinical role of the TME in solid cancer. British Journal of Cancer, 2019, 120 (1), pp.45-53. 10.1038/s41416-018-0327-z. hal-01985551

HAL Id: hal-01985551
https://hal.sorbonne-universite.fr/hal-01985551
Submitted on 18 Jan 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
The highly complex and heterogenous ecosystem of a tumour not only contains malignant cells, but also interacting cells from the host such as endothelial cells, stromal fibroblasts, and a variety of immune cells that control tumour growth and invasion. It is well established that anti-tumour immunity is a critical hurdle that must be overcome for tumours to initiate, grow and spread and that anti-tumour immunity can be modulated using current immunotherapies to achieve meaningful anti-tumour clinical responses. Pioneering studies in melanoma, ovarian and colorectal cancer have demonstrated that certain features of the tumour immune microenvironment (TME)—in particular, the degree of tumour infiltration by cytotoxic T cells—can predict a patient’s clinical outcome. More recently, studies in renal cell cancer have highlighted the importance of assessing the phenotype of the infiltrating T cells to predict early relapse. Furthermore, intricate interactions with non-immune cellular players such as endothelial cells and fibroblasts modulate the clinical impact of immune cells in the TME. Here, we review the critical components of the TME in solid tumours and how they shape the immune cell contexture, and we summarise numerous studies evaluating its clinical significance from a prognostic and theranostic perspective.

INTRODUCTION

The tumour microenvironment (TME) is a highly complex ecosystem. Tumour cells co-exist with immune cells (including macrophages, polymorphonuclear cells, mast cells, natural killer cells, dendritic cells (DCs), and T and B lymphocytes) and non-immune cells (such as endothelial cells and stromal cells) and establish subtle interactions with them that determine the tumour’s natural history. In particular, the immune cell component of a tumour is fundamental in determining the tumour’s fate, and its invasive and metastatic ability. A large variety of immune cells can infiltrate tumours, and their composition and organisation within the TME are tightly associated with the clinical outcome of cancer patients. Current efforts to include immune parameters among the classical oncology prognostic classification tools have shown promising results.

The advancements in our understanding of the TME have also led, in recent years, to the development of efficacious therapies to treat advanced cancer. The treatment of thousands of cancer patients with monoclonal antibodies targeting inhibitory receptors expressed by immune cells (immune checkpoint blockade) has yielded remarkable response rates in several types of solid and haematologic malignancies. In this context, the analysis of the TME has become fundamental to predict response to treatment.

The constantly evolving knowledge about the complexity of the cancer niche and the dynamic interactions between all its components has changed the way we think about tumours. In this integrated model, the TME is shaped in situ by tumour and non-tumour cellular components, and by other elements such as the surrounding microbiota. In this article, we discuss evidence that supports the immune microenvironment as an essential player in this integrated cancer model, and we summarise numerous studies evaluating its clinical significance from a prognostic and theranostic perspective in solid tumours.

THE TME

The neoplastic immune microenvironment is extremely complex, as virtually all immune cell types, including macrophages, polymorphonuclear cells, mast cells, NK cells, DCs, and T and B lymphocytes, can infiltrate cancer tissues. The role of these immune cell types in tumour evolution and growth is diverse and is tightly linked to their inherent functions and to the molecules they express (e.g., cytokines or inhibitory ligands).

The prototypical anti-tumour immune cell is the CD8+ T lymphocyte, which can recognise tumour cells in an antigen-specific manner and secrete cytotoxic molecules to kill them directly. Before exerting their cytotoxic functions, however, CD8+
T cells must be primed and educated by professional antigen presenting cells (APCs), i.e., DCs. Although these interactions between naive T cells and mature DCs cells have traditionally been thought to take place in secondary lymphoid organs (i.e., lymph nodes), it is now clear that they can also occur within, or adjacent to, the tumoural tissue, in organised tertiary lymphoid structures. These specialised structures provide an area within the TME that is protected from the immunomodulatory effects of the tumour or stromal cells, and is enriched with T cell activation cytokines. Recent studies suggest that the anti-tumour immune response can be orchestrated within these structures. Cytotoxic NK cells can also exert anti-tumour killing independently of any previous interaction with APCs, notably in the case of a loss of class I major histocompatibility complex (MHC) molecules on the surface of tumour cells. CD4+ Th1-oriented T cells are also important in promoting an anti-tumour immune response through the production of cytokines essential for T cell proliferation, as well as for macrophage recruitment and activation.

By contrast, other immune cells, such as tumour-promoting M2 macrophages and immature granulocytic and monocytic cells (myeloid-derived suppressor cells (MDSCs)) can favour tumour progression through the induction of stromal cell proliferation, vascularisation, extracellular matrix deposition (ECM), and cell migration. These and other immune cells can also promote tumour progression by inhibiting the in situ immune response. The prototypical immunosuppressor cells are regulatory CD4+ T lymphocytes (Treg), which directly secrete or facilitate the formation of immunosuppressive molecules (e.g., IL-10, adenosine), and modulate the APC function (e.g., via CTLA-4–CD80/86 interactions).

Some immune cells often demonstrate plasticity in the TME, showing both tumour-promoting and tumour-inhibiting potential. For example, whereas some macrophages (M1) mainly produce pro-inflammatory cytokines that potentiate the anti-tumour immune response, others (M2) can promote fibroblast proliferation, ECM deposition and immunosuppression. Importantly, the total composition of immune cells in the TME is not a binary ‘anti-tumour’ or ‘pro-tumour’ environment, but rather a mixture of these cell types. The overall phenotype is determined by the interactions of the immune cells with each other and with non-immune cells. The tumour and stromal cells play an essential role in determining the composition of the immune cell infiltrate, as well as the rate of recruitment, and their in situ phenotype and function.

**SHAPING THE TME**

The tumour mutational landscape

Genetic mutations, ranging from single base substitutions to chromosome translocations, are the cornerstone of the development of neoplastic lesions. Individual DNA mutations that accumulate over time often lead to the stepwise transformation of normal cells into dysplastic and eventually neoplastic tumour cells. These mutations can be induced by a broad array of cellular stressors, such as carcinogens in cigarette smoke, ionising radiation including UV light from the sun, or reactive oxygen species generated by myeloid cells during chronic inflammation.

The number and characteristics of these pathogenic tumour mutations shape the composition of the immune microenvironment through several different mechanisms. First, a portion of mutated peptide epitopes resulting from either driver or passenger mutations can be presented on tumour cell class I MHC molecules and be recognised by CD8+ T cells, which, together with T-cell-attracting chemokines such as CXCL9 and CXCL10, promotes a brisk infiltration by cytotoxic T lymphocytes. The mutations that create tumour-associated neoantigens have become more appreciated with recent advances in sequencing and computational techniques, leading to the concept that a tumour mass is often composed of different subclones of cells with different immunogenic potentials. The prototypical example of immune cell response induced by tumour cell mutations is microsatellite instable (MSI) colorectal cancer.
Recruitment of mature immunosuppressive neutrophils and breast, lung and gastrointestinal cancer, also activate granulopoiesis. It has been shown that other primary tumours, including those in the pancreatic adenocarcinoma and renal cell carcinoma (RCC), have a desmoplastic reaction, which represents a physical barrier for the movement of immune cells. The extent of T cell infiltration in these tumours is tightly correlated with neoantigen frequency. However, no such correlation has been observed in melanoma patients. Other tumours, such as pancreatic adenocarcinoma and renal cell carcinoma (RCC), have a lower frequency of neoantigens but might still exhibit a high degree of infiltration by T cells (Fig. 2). Thus, although the mutational load is tightly correlated with the degree of tumour inflammation, the nature of the driver mutations and additional neoantigenic mutations that accumulate critically influence the nature and function of the immune cell composition.

**Fig. 2** The tumour microenvironment and mutational landscape across tumour types. Heatmap representing the average number of somatic mutations (ranging from 0.7 per megabase in thyroid cancer, to 11 per megabase in melanoma) and relative abundance of infiltrating immune and stromal cells across 15 different human tumours as determined by Microenvironment Cell Populations-counter (MCP-counter).

(CRC). This subtype represents ~15% of CRC cases and is characterised by defective DNA mismatch repair machinery, resulting in an increased rate of mutagenesis as compared with microsatellite stable tumours (MSS). Furthermore, MSI CRC tumours display increased infiltration with CD8+ T cells, B cells and macrophages, as well as an increased expression of Th1-related genes.

Second, some driver or passenger mutations can induce molecular pathways that shape tumour infiltration by immune cells independently of their neoantigenic potential. For example, the mutation-driven activation of the Wnt-β-catenin pathway in melanoma, colorectal cancer and hepatocellular carcinoma limit the accumulation of cytotoxic T cells and DCs. In addition, models of lung adenocarcinoma suggest that mutations in the Myc and Ras pathways cooperate to establish an immunosuppressive microenvironment by driving expression of the chemokine CCL9—which recruits immunosuppressive and angiogenic macrophages—and interleukin (IL)-23, and prevents the accumulation of cytotoxic NK cells and T cells in the tumour. Similarly, KRAS mutations in pancreatic ductal cells drive the expression of granulocyte-macrophage colony-stimulating factor, which leads to the recruitment of large numbers of neutrophils and inhibits the accumulation of cytotoxic T cells and NK cells. These processes result in an increased rate of mutagenesis as compared with microsatellite stable tumours (MSS). Furthermore, MSI CRC tumours display increased infiltration with CD8+ T cells, B cells and macrophages, as well as an increased expression of Th1-related genes.

Third, mutations in tumour cells can alter immune cell functions once they are recruited within the tumour mass. For example, Coelho et al. found that oncogenic RAS can drive tumour cell-intrinsic upregulation of the programmed cell death ligand 1 (PD-L1). By binding to programmed cell death protein 1 (PD-1) expressed on activated T cells, PD-L1 suppresses the effector activity of T cells that might otherwise confer cytotoxicity. These three mechanisms shape the composition of immune cells within the tumour significantly. In some tumour types, such as lung adenocarcinoma, the extent of T cell infiltration is tightly correlated with neoantigen frequency. However, no such correlation has been observed in melanoma patients. Other tumours, such as pancreatic adenocarcinoma and renal cell carcinoma (RCC), have a lower frequency of neoantigens but might still exhibit a high degree of infiltration by T cells (Fig. 2). Thus, although the mutational load is tightly correlated with the degree of tumour inflammation, the nature of the driver mutations and additional neoantigenic mutations that accumulate critically influence the nature and function of the immune cell composition.

**The non-immune components**

Tumour cells have high mitotic and metabolic rates and therefore need to maintain sufficient oxygen and nutrient levels to support their growth. A vascular network is fundamental for a tumour’s development. The hypoxic tumour environment promotes the production of proangiogenic factors (e.g., vascular endothelial growth factor, transforming growth factor-β, fibroblast growth factor and platelet-derived growth factor), which can prompt rapid angiogenesis but often results in the formation of aberrant vasculature. Several studies have shown that tumour-associated endothelial cells often express low levels of leucocyte adhesion molecules (e.g., ICAM1 and VCAM1) and T cell-recruiting chemokines, thereby impeding the recruitment of anti-tumour immune cells. Furthermore, the tumour blood vessels are tortuous, leaky and with reduced pericyte coverage, which might present mechanical barriers to infiltration by T cells.

Conversely, in situations where the tumour vasculature does contain appropriate ligands, an active T cell infiltrate can be present. Of particular interest is the development of tertiary lymphoid structures (TLSs), which are highly specialised immune aggregates. These immune aggregates contain not only a high density of immune cells facilitating their interactions but also a highly efficient and specialised vasculature (so-called ‘high endothelial venules’), which are otherwise only found in lymph nodes. These specialised vessels are capable of actively recruiting naïve T cells and B cells from the circulation. Mature DCs that migrate to the site activate and educate the newly recruited T cells, which proliferate and differentiate into T effector memory cells. The B cells also proliferate and differentiate into plasma cells. Thus, although many characteristics of the tumour vasculature hinder the development of an immune cell composition that is predominantly ‘anti-tumour,’ certain cases, such as the formation of TLSs and infiltration by T and B cells, can support an adaptive anti-tumour immune response.

Of the other non-endothelial tumour-associated stromal cells, fibroblasts have been most convincingly shown to shape the process of immune cell recruitment and differentiation. Various studies suggest that under certain stimuli, cancer-associated fibroblasts (CAF) can acquire a pro-inflammatory signature characterised by the expression of immunomodulatory molecules (e.g., TGF-β or PD-L1/L2), as well as chemokines that promote recruitment of immunosuppressive myeloid cells (e.g., CXCL12, CCL2, CCL3, CCL4 and CCL5). In addition, CAF often form part of an intricate and thick arrangement of cells and stromal matrix surrounding tumour nests (also known as a desmoplastic reaction), which represents a physical barrier for cytotoxic immune cell infiltration.
Finally, by shaping the balance of nutrients and oxygen within the TME, the tumour cell can also actively modulate the phenotype and function of the infiltrating immune cells. Several studies have provided evidence that the function of the immune cells, in particular T cells, is tightly linked to their metabolic state and the abundance of certain nutrients. For instance, the depletion of glucose or arginine impair T cell proliferation and cytotoxic functions. The consumption of nutrients and the expression of certain enzymes by the tumour cells can thus deeply impact the TME. Chang et al. showed that the consumption of glucose by tumour cells metabolically restricts the infiltrating T cells, leading to dampened IFN-γ production. Also, the overexpression of indoleamine 2,3-dioxygenase (rate-limiting enzyme of tryptophan catabolism) by the tumour cells has been associated with the inhibition of T cell functions.

The unexpected players: the gut microbiota
Recent evidence has highlighted that the microbiota can have a significant effect on the rate of tumour growth and spread, probably by shaping the systemic and in situ immune response. Within the human body, several organs—including the skin, gut and other mucosas—are colonised by several trillions of microbes, which constantly interact with the host. This interaction has relevant systemic effects, including shaping the functional diversity and the repertoire of B and T cells. Although certain commensal bacteria seem necessary to maintain a tonic baseline immune response, others can induce a systemic immunoregulatory effect. For example, alterations in the gut microbiota are associated with HIV disease progression. In contrast, several studies suggest that certain bacteria can induce a local and systemic expansion of Treg that are essential to prevent tissue inflammation.

Not surprisingly, alterations in the gut microbiota also have effects on cancer development and spreading. For instance, it has been shown that hepatocellular carcinoma is promoted by the intestinal microbiota via activation of TLR4. The area with the most interesting evidence is currently cancer therapeutics. In mice, two studies showed that chemo- and radiotherapy promoted gastrointestinal bacterial translocation into the systemic circulation, which probably boosts the immune response and promotes post-treatment tumour rejection. Similarly, Lida et al. showed that the CD8+ T cell-driven tumour rejection induced by intratumoural CpG-oligodeoxynucleotides is inhibited by antibiotics.

In the area of checkpoint blockade, recent studies have shown promising evidence that the response to these agents is modulated by the gut microbiota. Vetizou et al. showed that the anti-tumour effects of CTLA-4 blockade depends on distinct Bacteroides species. In this study, tumours in antibiotic-treated or post-therapy tumour rejection. Similarly, Lida et al. showed that the CD8+ T cell-driven tumour rejection induced by intratumoural CpG-oligodeoxynucleotides is inhibited by antibiotics.

In the area of checkpoint blockade, recent studies have shown promising evidence that the response to these agents is modulated by the gut microbiota. Vetizou et al. showed that the anti-tumour effects of CTLA-4 blockade depends on distinct Bacteroides species. In this study, tumours in antibiotic-treated or post-therapy tumour rejection. Similarly, Lida et al. showed that the CD8+ T cell-driven tumour rejection induced by intratumoural CpG-oligodeoxynucleotides is inhibited by antibiotics.

THE IMMUNE CELL CONTEXTURE AS A PROGNOSTIC TOOL IN MODERN CLINICAL PRACTICE
The analysis of the immune microenvironment in retrospective cohort studies across different tumours has established a clear correlation between the density of infiltrating immune cells and the patient's clinical outcome. More than 280 articles assessing the correlation between the presence of distinct immune cell populations and patient prognosis have been published to date (reviewed in detail in ref. 74-76). Overall, clear-cut evidence has established that the presence of the main cellular players orchestrating the cytotoxic anti-tumour immune response (e.g., cytotoxic CD8+ T cells, Th1-oriented CD4+ T cells, mature activated DCs and TLSs) is associated with a good clinical outcome in the vast majority of tumour types. In contrast, high densities of macrophages—specifically M2-oriented—and Tregs are associated with poor prognosis. The major efforts to include quantification of these immune populations in the standard clinical practice have been conducted in melanoma and colorectal cancer, as outlined below.

There are a few rare examples of tumours that do not follow the association between high infiltration with CD8+ T cells and a positive prognosis. RCC has been the best studied of these examples to date, and a relevant number of studies suggest that increased densities of CD8+ cells are associated with patients’ shorter survival. Recent evidence highlights that this unexpected correlation is probably related to a dysfunctional immune cell response in this tumour type, and to the expression of inhibitory receptors by tumour-infiltrating T cells. In node-positive prostate cancer, a stronger infiltration by CD8+ T cells has also been associated with an enhanced risk of metastasis. This paradoxical association has also been described in some haematologic malignancies (e.g., diffuse large B-cell lymphoma and Hodgkin lymphoma).

Melanoma
Melanoma was one of the first tumour types in which a high density of tumour-infiltrating lymphocytes (TILs) was found to correlate with favourable patient prognosis, including a lower incidence of lymph node metastasis and longer disease-free survival (DFS). Pioneering studies by Azimi et al. and Thomas et al. generated data from >4000 patients with melanoma, semi-quantitatively grading their lesions according to the degree of lymphocyte infiltration on Hematoxylin and eosin-stained slides. This grading scheme (grade 0, TILs absent; grade 1, either a mild or moderate focal or a mild multifocal lymphocyte infiltrate; grade 2, a marked focal, either a moderate or marked multifocal, or a mild diffuse lymphocyte infiltrate; and grade 3, a moderate or marked diffuse lymphocyte infiltrate) was an independent predictor of DFS, such that a lower grading correlated with a lower DFS. Notably, patients with tumours assessed to be TIL grade 3 showed 100% survival after 5 years. These and other studies have helped the TIL grading system win recognition among clinicians as a feasible and inexpensive prognostic factor in patients with melanoma, and it is now routinely reported by pathologists following the recommendations of the College of American Pathology (cancer protocol templates for melanoma). This approach supplements other pathologic parameters that predict patient prognosis, including tumour, node and metastasis (TNM) staging, the presence of vascular invasion or tumour regression and the mitotic rate.

Colorectal cancer
Another big effort to validate the prognostic significance of the presence of TILs has been led in CRC, in which an immunohistochemistry (IHC)-based grading score system called Immuno-score has been optimised and developed. This process involves scanning a stained slide and analysing it using digital pathology IHC quantification software. Under the Immunoscore grading system, CD3+ and CD8+ cells in the invasive margin and the internal core of the tumour are quantified (as cells/mm²) and scored as low, intermediate, or high. These groups were defined using cell density cutoffs based on the mean distribution of CD3+.
Contrary to most tumours in which increased numbers of CD8+ T cells are associated with a favourable clinical outcome, the opposite association has been described for RCC. Our research group has studied this paradoxical association in detail. Our data suggested that the increased infiltration with CD8+ T cells in RCC can be accompanied by either a well orchestrated or an immunosuppressive TME, and this context determines the patient’s prognosis. Although two-thirds of the tumours with high densities of CD8+ TIL are associated with low infiltration by mature DCs, increased expression of inhibitory ligands (e.g. PD-L1 and PD-L2) and poor clinical outcome, the remaining third is highly enriched in TLSs, exhibit proliferating T cells and is associated with remarkably long PFS.97,98

Altogether these data supported the existence of three different immune profiles in RCC: one with activated fully functional T cells (immune-activated); one with abundant but inhibited T cells (immune-inhibited); and one with low infiltration by any immune cell type (immune-silent).2,93,94 To prove this concept, we used multiparametric flow cytometry to analyse the phenotype of tumour-infiltrating T cells in 38 patients with clear cell renal carcinoma (ccRCC) (T stage 1–3), and followed the patients prospectively for one year (median follow-up 11 months ± 6).78 We investigated the co-expression of 14 activation molecules and inhibitory receptors and subclassified the tumours by unsupervised methods using phenotype data. We corroborated the existence of three immune profiles in RCC and determined that patients with immune-inhibited tumours had an extremely high risk of recurrence in the first year after surgery (70%). These tumours were characterised by the presence of abundant regulatory CD4+ T cells and CD8+ T cells co-expressing several inhibitory receptors (e.g., PD-1, Tim-3 and Lag-3). We have recently updated the PFS data for this prospective cohort (median follow-up of 26 months ± 6, Fig. 3) and confirmed the significant differences in the PFS between patients with these three groups of tumours. The median survival of patients with immune-inhibited tumours was only 8 months, but it has not yet been ascertained for individuals with immune-silent or immune-activated tumours (Fig. 3).

We also analysed phenotypic T cell markers in the peripheral blood lymphocytes of this group of ccRCC patients and, through unsupervised methods, were able to define two main groups of patients: peripheral blood lymphocyte (PBL)-immune-silent, with almost absent expression of activation markers (e.g., CD69 and inducible T cell co-stimulator) or inhibitory receptors (e.g., PD-1, Tim-3 and CTLA-4); and PBL-immune-inhibited, with prominent expression of activation markers and inhibitory receptors. The updated follow-up of these patients showed a sharp difference in their PFS (Fig. 3). Although the disease has progressed in almost 80% of the patients with PBL-immune-inhibited after 24 months, this number only reaches 10% in the PBL-immune-silent group. This is a relevant finding given the feasibility of analysing the expression of phenotypic markers in PBL from cancer patients. These promising results are currently being investigated in prospective clinical trials to evaluate its significance as prognostic and theranostic tools.

Other tumours

Although not always exhaustively studied in the clinical setting, other solid malignancies deserve particular attention given the abundant evidence associating the TME with clinical outcome.

In breast cancer, the analysis of thousands of samples has found a strong association between high infiltration with CD8+ T cells or a Th1-gene signature and longer PFS and OS.95,100 Also, it has been suggested that this association is particularly strong in oestrogen receptor (ER) negative, HER-2 negative, as well as ER,
programmed death ligand 1 (PD-L1) and programmed death receptor 1 (PD-1) in cancer. The quantification of tumour mutational loads has been included in some clinical trials evaluating the activity of PD-1/PD-L1 blockade and has revealed its potential association with therapeutic response. In patients with NSCLC and treated with pembrolizumab, some tumoural non-synonymous mutations above the cohort’s median have been associated with higher ORR and longest PFS. Interestingly, these studies have also suggested that patients with PD-L1 tumours and high mutational burdens exhibit the highest sensitivity to anti-PD-1 therapy (91%). These findings were supported by a clinical trial in urothelial cancer where the activity of atezolizumab was tested; responding patients exhibited two-fold higher mutational loads than non-responding patients. Finally, a study in melanoma-bearing patients receiving pembrolizumab (n = 38) found that high mutational burdens, although not associated with clinical response, correlated with improved survival.

Density of TIL
Some clinical trials have also evaluated the correlation between TIL density and clinical response to anti-PD-1/PD-L1 therapies. Chen et al. reported higher densities of CD8+ TILs in CD45RO+ memory TIL in the pre-treatment biopsies from patients with melanoma who responded to nivolumab than non-responders. A similar association between CD8+ TIL numbers and response to treatment has been reported in patients with melanoma and colorectal cancer treated with pembrolizumab or atezolizumab. By contrast, two independent studies have reported that CD8+ TIL densities are not associated with response to anti-PD-1 in metastatic RCC.

CONCLUSIONS
During the past two decades our understanding of the complexity of the TME has increased considerably. In-depth characterisation of each of the cellular components in cancer has shed light on the convoluted network of interactions between the numerous components within a tumour mass. Furthermore, the clinical follow-up of large cohorts of cancer patients has shown how these interactions have largely determined the clinical evolution of cancer. The addition of immune-based prognostic biomarkers to the current clinical practice of oncology and pathology is imminent, as it rapidly gains recognition within the medical community. The next generation of tumour histology-based predictive biomarkers will probably transcend single-stain HCs, as the whole research field is rapidly moving toward multiparametric and highly complex techniques (i.e., multispectral immunofluorescence, spatial transcriptomics and mass-spectrometry-based tissue imaging.). The challenge facing the tumour immunology community is how to translate these new highly complex findings into relevant, simple and consistent biomarkers to use in the clinical setting.

ACKNOWLEDGEMENTS
This work was supported by the Institut National de la santé et de la Recherche Médicale (INSERM), University Paris-Descartes, University Pierre and Marie Curie, the Site de Recherche Intégrée sur le Cancer (SIRIC) Cancer Research for Personalised Medicine (CARPEM) programme, the LabEx Immuno-Oncology (LAEX62_S1UMR5792).
FRIDMAN, the Institut National Du Cancer (INCa), the Cancéropôle Ile-de-France, O. Lecomte. Florent Petitprez is recipient of a CARPEM fellowship. Yann Vano was supported by a CARPEM fellowship.

AUTHOR CONTRIBUTIONS
All the authors contributed to the conception, drafting and final revision of the manuscript.

ADDITIONAL INFORMATION
Competing interests: The authors declare no competing interests.

Consent for publication: All authors read and approved the manuscript.

Note: This work is published under the standard license to publish agreement. After 12 months the work will become freely available and the license terms will switch to a Creative Commons Attribution 4.0 International (CC BY 4.0).

REFERENCES
1. Giraldo N. A., Taube J.M. PD-L1 and Other Immunological Diagnosis Tools. In: Zitvogel L., Kroemer G. (eds) Oncoimmunology (Springer, Cham, 2018).
2. Giraldo, N. A. et al. The immune contexture of primary and metastatic human tumours. Curr. Opin. Immunol. 27, 8–15 (2014).
3. Dranoff, G. Cytokines in cancer pathogenesis and cancer therapy. Nat. Rev. Cancer 4, 11–22 (2004).
4. de Chaisemartin, L. et al. Characterization of chemokines and adhesion molecules associated with T cell presence in tertiary lymphoid structures in human lung cancer. Cancer Res. 71, 6391–6399 (2011).
5. de Chaisemartin, L. et al. Characterization of chemokines and adhesion molecules associated with T cell presence in tertiary lymphoid structures in human lung cancer. Cancer Res. 71, 6391–6399 (2011).
6. Goc, J. et al. Dendritic cells in tumor-associated tertiary lymphoid structures signal a Th1 cytokine immune contexture and license the positive prognostic value of infiltrating CD8+ T cells. Cancer Res. 74, 705–715 (2014).
7. Moran, M. G. & Lanier, L. L. NK cells and cancer: you can teach innate cells new tricks. Nat. Rev. Cancer 16, 7–19 (2016).
8. Haabeth, O. A. W. et al. Inflammation driven by tumour-specific Th1 cells protects against B-cell cancer. Nat. Commun. 2, 240 (2011).
9. Qian, B.-Z. et al. CCL2 recruits in...
52. Cham, C. M. & Gajewski, T. F. Glucose availability regulates IFN-γ production and p70S6 kinase activation in CD8+ effector T cells. J. Immunol. 174, 4670–4677 (2005).
53. Fletcher, M., et al. I-AgRine depletion blunts antitumor T cell responses by inducing myeloid-derived suppressor cells. Cancer Res. 75, 273–283 (2014).
54. Chang, C.-H. et al. Metabolic competition in the tumor microenvironment is a driver of cancer progression. Cell 162, 1229–1241 (2015).
55. Uyttenhove, C. et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nat. Med. 9, 1269–1274 (2003).
56. Honda, K. & Littman, D. R. The microbiota in adaptive immune homeostasis and disease. Nature 535, 75–84 (2016).
57. Bouskra, D. et al. Lymphoid tissue genesis induced by commensals through TLR4. Cell 154, 550–564 (2013).
58. Viaud, S. et al. The intestinal microbiota modulates the anticancer immune response in murine tumors. Cell 154, 550–564 (2013).
59. Becht, E., Giraldo, N. A., Dieu-Nosjean, M.-C., Sautès-Fridman, C. & Fridman, W. H. The immune contexture in cancer prognosis and treatment. J. Clin. Oncol. 31, 2705–2713 (2013).
60. Park, M. H., Lee, J. S. & Yoon, J. H. High expression of CXCL1 by tumor cells correlates with a good prognosis and increased tumor-infiltrating CD8+ T cells, natural killer cells, and dendritic cells in breast carcinoma. J. Surg. Oncol. 106, 386–392 (2012).
61. de Kruif, E. M. et al. Tumor immune subtypes distinguish tumor subclasses with clinical implications in breast cancer patients. Breast Cancer Res. Treat. 142, 355–364 (2014).
62. Mohammed, Z. M. A., Going, J. J., Edwards, J., Elsberger, B. & McMillan, D. C. The relationship between lymphocyte subsets and clinico-pathological determinants of survival in patients with primary operable invasive ductal breast cancer. Br. J. Cancer 109, 1676–1684 (2013).
63. de Kruif, E. M. et al. Tumor immune subtypes distinguish tumor subclasses with clinical implications in breast cancer patients. Breast Cancer Res. Treat. 142, 355–364 (2014).
64. Mahmoud, S. M. A. et al. Tumour-infiltrating macrophages and clinical outcome in breast cancer. Br. J. Pathol. 9, 159–163 (2012).
65. Hiraoka, K. et al. Concurrent infiltration by CD8+ T cells and CD4+ T cells is a favourable prognostic factor in non-small-cell lung carcinoma. Br. J. Cancer 94, 275–280 (2006).
66. Kawai, O. et al. Predominant infiltration of macrophages and CD8(+) T cells in cancer nests is a significant predictor of survival in stage IV nonsmall cell lung cancer. Cancer 113, 1387–1395 (2008).
67. Ruffini, E. et al. Clinical significance of tumor-infiltrating lymphocytes in lung neoplasms. Ann. Thorac. Surg. 87, 365–371 (2009).
68. Klic, A., Landreneau, R. J., Luketic, J. D., Pennathur, A. & Schuchert, M. J. Density of tumor-infiltrating lymphocytes correlates with disease recurrence and survival.
