Control of Dendritic Cell Function Within the Tumour Microenvironment

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The tumour microenvironment (TME) presents a major block to anti-tumour immune responses and to effective cancer immunotherapy. The inflammatory mediators such as cytokines, chemokines, growth factors and prostaglandins generated in the TME alter the phenotype and function of dendritic cells (DCs) that are critical for a successful adaptive immune response against the growing tumour. In this mini review we discuss how tumour cells and the surrounding stroma modulate DC maturation and trafficking to impact T cell function. Fibroblastic stroma and the associated extracellular matrix around tumours can also provide physical restrictions to infiltrating DCs and other leukocytes. We discuss interactions between the inflammatory TME and infiltrating immune cell function, exploring how the inflammatory TME affects generation of T cell-driven anti-tumour immunity. We discuss the open question of the relative importance of antigen-presentation site; locally within the TME versus tumour-draining lymph nodes. Addressing these questions will potentially increase immune surveillance and enhance anti-tumour immunity.

Keywords: tumour microenvironment (TME), inflammatory cytokines, dendritic cells, anti-tumour immunity, draining lymph nodes, Tertiary Lymphoid Structures (TLS), immune infiltration

INTRODUCTION

Anti-tumour immunity is the ability of the body’s immune system to recognise and eliminate tumour cells. This phenomenon has the potential to cure cancer even if cells are widely disseminated through multiple metastatic sites and has been harnessed to develop different immunotherapy drugs. With increased understanding of immune surveillance process by innate immune cells and discovery of T cell immune checkpoints, such as PD-1, PD-L1, and CTLA-4; cancer immunotherapy has significantly improved patient survival and quality of life (1–5). Treatments aim to promote successful infiltration and activation of antigen presenting cells and boost T-cells cytotoxic activity to promote anti-tumour immunity. However, despite promising results, not all tumour types or patients respond equally to immunotherapy (6–8). The major reasons for failure of immunotherapy are (1) reduced antigenicity (9–11) and (2) immunosuppressive tumour microenvironment (TME) (12–15). The TME is highly heterogeneous; consisting of tumour cells, stromal cells, extracellular matrix (ECM) and immune cell types including macrophages, dendritic cells, T and B lymphocytes, Natural killer (NK) cells, mast cells, myeloid derived suppressor cells

Abbreviations: APC, Antigen Presenting cells; LNs, Lymph nodes; TDLN, Tumour draining lymph node; TME, Tumour microenvironment; DCs, Dendritic cells; PGE2, Prostaglandin E2; ECM, Extracellular matrix; CAF, Cancer associated fibroblasts; TLS, Tertiary lymphoid structures.
DC Maturation and DC Gene Signatures in Tumours

DCs are the professional APCs responsible for activation and maintenance of tumour-specific cytotoxicity by T cells (28, 29). Tumour infiltrating conventional DCs (cDC1 and cDC2) scan and phagocytose tumour antigens (30–32); and subsequently migrate to secondary lymphoid tissues to prime naïve CD8+ T cells (31). However, immune surveillance by APCs and T-cell infiltration can be impaired by dynamic changes within the tumour microenvironment such as induction of chemokines, cytokines, growth factors, inflammation, ECM modulators and immune checkpoint proteins (22–27). This review focuses on the immunosuppressive properties of the TME and how these mechanisms alter activation, maturation and trafficking of dendritic cells to enable immune escape and tumour progression.

TABLE 1 | Tumour infiltrating DC subsets detected in various human solid tumours – Liver, Ovarian, Lung, Breast and Colorectal (69, 70).

| DC subsets                 | Markers                      |
|---------------------------|------------------------------|
| cDC1                      | XCR1, CLEC9A, CADM1, CD141, CD103 |
| cDC2                      | CD11b, SIRPa, CLEC10A, FCER1A, CD1c |
| mDC3                      | MARCKS, CCL19, LAMP3, BATF3, CCR7, CD40 |
| pDC                       | TC4, CCR6, IL7RA, CLEC4C, IFN7 |
| DC5 or inflammatory DCs   | CD1c, CD201, CD1A, CD14 |

...powerful tool to map tumour-driven immune changes and to design future immune therapies leveraging DC biology. scRNA-seq studies on various human tumours, including non–small cell lung cancer (NSCLC) (59–62), head and neck squamous cell carcinoma (63), hepatocellular carcinoma (64), melanoma (65, 66), cutaneous squamous cell carcinoma (67), colorectal cancer (68, 69), ovarian cancer (61), and breast cancer (61) have identified tissue-specific DC subsets as well as those conserved across cancer types. By comparing tumour infiltrating DC states across various tumour studies, five major DC subsets have been defined that are conserved in most tumour types (69, 70) (Table 1). Four major ones are cDC1, cDC2, migratory DC3 (mDC3) and plasmacytoid DC (pDC); and the DC subset (DC5) that were less conserved, mostly contained cDC2 state (CD1C+) but additionally either expressed Langerhans cell-specific markers (CD201, CD1A) or monocyte markers (CD14, CD11b) such as in case of NSCLC (61, 62, 69, 70). DC5 were also referred as inflammatory DCs as these have phenotypic similarities to monocytes but are functionally different due to their cDC2-specific antigen presentation properties (71). On the other hand, classical monocytes (CD14+ CD16-) play a key role in tissue homeostasis and inflammation (72). Like monocytes, inflammatory DCs are also capable of releasing TNF-α and inducible nitric oxide synthase (iNOS) upon pathogen recognition. In addition, there is a subset of cDCs that induce antigen-specific tolerance in lymph nodes (LNs) known as regulatory DCs (DCregs) (73, 74). These are characterized by low MHC expression and therefore weaker antigen presentation capability to effector T cells. Instead, they can induce proliferation of regulatory T cells (Tregs) resulting in immune tolerance. These properties have led the use of DCregs in organ transplantations (75).

Overall cDC2 phenotype is the most abundant, while the other DC subtypes vary in each cancer type (61, 76). Single cell sequencing and clustering analysis have identified transcription factors underlying each DC phenotype, including BATF3 for cDC1s, CEBPB for cDC2s, NFkB2 for migratory DCs and TCF4 for pDCs (61, 77). Another study reports differential expression of costimulatory molecules and immune checkpoints on different DC subsets present in the TME (78). Although these phenomena are tightly regulated, heterogeneity of TME can influence the transcriptional factor activity, expression of costimulatory molecules and hence DC maturation and/or migration (78–82). This new in-depth knowledge of DC gene signatures can facilitate the design of a favourable antitumour response or identification of response biomarkers for targeted therapies (83).
**TME FACTORS AFFECTING DC DEVELOPMENT IN TUMOURS**

**Pro- and Anti-Inflammatory Factors**

The immunosuppression of tumour-infiltrating DCs can be facilitated by various soluble factors secreted in the TME such as IL-6, IL-10, IDO, M-CSF, transforming growth factor-β1 (TGF-β1), PGE2, VEGF (Figure 1) (84–91); although promisingly some of these defects in DC development or function have been proven to be reversible in pre-clinical models and clinical trials (27, 91–94). Mature DC numbers or functions were improved leading to better immune control of the tumour in several mouse models: IL-6 KO mice (95); tumours treated with anti-VEGF antibody (96, 94). Mature DC numbers or functions were improved leading to better immune control of the tumour in several mouse models: IL-6 KO mice (95); tumours treated with anti-VEGF antibody (96, 94); and treatment with anti-IL-8 monoclonal antibody (98, 99).

On the other hand, pro-inflammatory cytokines such as IFN-α, IL-2, IL-15, IL-21 and GM-CSF are also present in the TME (Figure 1) that contribute to enhanced antigen priming, improved DC maturation and increased immune infiltration in tumours (100–103). Therefore, the complex balance of inflammatory signals in the TME is an area of intense research interest but is not trivial to target currently. One of the recent studies on human melanoma reported the correlation of pro-inflammatory cytokine FLT3L production (by NK cells) with abundant intratumoral stimulatory DCs, improved patient responsiveness to anti-PD-1 therapies and better overall survival (104).

The inflammatory factors described above can be derived from tumour cells, immune cells or stromal cells such as fibroblasts surrounding tumour (61, 88, 105, 106). Various subtypes of fibroblasts based on different tissue specific identity, localization, function, transcription factor expression, collagen factors, cancer hallmark genes etc. make up the total tumour mass. CAFs or cancer associated fibroblasts represent a major population in the TME of many solid tumours, however their origin and role in tumour progression is complex and they can generate pro-tumourigenic and anti-tumourigenic secretory factors. Phenotypically and functionally different CAF subtypes based on cell-surface markers such as podoplanin (PDPN), α-smooth muscle actin (αSMA), fibroblast-activated protein (FAP), fibroblast-specific protein-1 (FSP-1/ S100A4), THY1 (also known as CD90), and platelet-derived growth factor receptor-α, and β (PDGFRα and PDGFRβ) have been associated with different tumour types, stages and patient survival (107–111). Recently, the ability of CAFs to modulate the immune responses has been discovered and is being explored to improve cancer therapies. CAFs also share some properties with fibroblasts in lymph nodes that already have a well-established role in DC migration (47, 112, 113); and therefore, parallels can be drawn between the two to better understand the DC trafficking in the TME. For example, PDPN present in fibroblasts interacts with CCL21 and promotes CCL21/CCR7 axis mediated DC migration in lymph node.

This knowledge was exploited to study the role of PDPN+ CAFs under the influence of hypoxia in tumour progression (114). The study reported PDPN overexpression due to hypoxia in fact favoured invasion of CCR7+ tumour cell into CCL21+ peripheral lymph nodes leading to metastasis (114, 115). Tumours associated with hypoxia are immunosuppressive and lack high expression of CCL21 and therefore therapeutic use of recombinant chemokines (such as CCL21) to stimulate immune cell recognition in tumours is being considered as a novel treatment approach (116, 117). Also, more research is required to understand the transition of a ‘normal’ fibroblast into an immunosuppressive phenotype such as S100A4+ PDPN+ CAFs as reported in breast cancer patients (109) or into an inflammatory CAF (iCAF) phenotype producing IL-6, IL-10, and IDO (118, 119) linked to poor patient survival. Authors of Fang et al. (118) have shown the role of the urokinase-type plasminogen activator, PLAU in conversion of fibroblasts to iCAFs in esophageal cancer (118), but much is still unknown about fibroblast differentiation in TME.

**TERTIARY LYMPHOID STRUCTURES (TLS)**

TLS are established at sites of chronic inflammation and can structurally and functionally resemble secondary lymphoid organs (120–122). Recent studies on murine models of TLS have shown the role of PDPN+ FAP+ immunofibroblasts in driving the development and expansion of TLSs (123, 124). These form part of the TME and can benefit from quick surveillance and locally primed immune response against tumour antigens (Figure 2). Occurrence of TLS correlated with high number of mature DCs, strong T-cell infiltration and long-term survival in human primary lung, breast, colorectal, melanoma and other tumours (120, 125–128). However, factors such as TLS location, tumour stage, tumour mutations, treatment history can affect immune cell infiltration and anti-tumour response (128, 129). The cells residing in TLS in tumours are known to express Th1, CD4, CD8, CD31, CD23, FOXP3, chemokines (CCL19, CCL21) and clusters of DC-Lamp+ mature dendritic cells (120, 130, 131) providing an immune-supportive niche (132–134). Typically, TLS at the periphery of the tumour have more organised and distinct DC/T-cell and B-cell zones than intratumoral TLS which contain mostly B cells (133). Future research understanding the immunological features of extratumoral versus intratumoral TLS will be useful to predict responsiveness to immunotherapy and overall survival.

**IMMUNE CHECKPOINT GENES**

The other group of molecules responsible for causing dysfunction in tumour-infiltrating DCs are immune checkpoint proteins PD-L1, PD-1, ILT2, CTLA4, TIM3 expressed by tumour cells or other immune cells (135–141). As mentioned before, expression of these inhibitory molecules is variable among DC subsets. For example, PD-1 and TIM-3 are mostly expressed on cDC1s; PD-1 expression specifically has been shown to inhibit NF-κB activation which is critical for DC functions including costimulatory molecule expression, antigen presentation and cytokine release leading to T cell inactivation (78, 135, 137, 139, 140). On contrary, ILT2 is expressed on pDCs and cDC2s, but not on cDC1s (78). The central goal of immunotherapies is inhibition of immune checkpoint genes and the expansion of mature cDCs and cytotoxic CD8+ T cells within tumours. It is associated with positive patient outcomes in multiple cancer types when combined with chemotherapy or radiotherapy treatments (28, 135, 142, 143). Despite this, many patients still fail to respond to immune checkpoint blockade. A
better understanding of the role of inflammatory mediators in determining tumour progression will also provide therapeutic avenues to improve immunotherapy outcomes (144–147).

Different labs have reported direct inhibition of pro-tumourigenic inflammation in combination with immune checkpoint blockade as a powerful strategy to improve the patient survival rates (27, 148–150). One such example is the use of aspirin that blocks the COX-2/PGE2 pathway and has shown promising results in preclinical melanoma models (27, 149). Prostaglandin E2 (PGE2), catalysed by the enzyme COX-2 is elevated in many
tumours (151) and plays a role in tumour evasion by directly inhibiting cytotoxic immune responses and subsequently mediates expression of other inflammatory molecules such as CXCL9, CXCL10, CXCR4, CXCL12, IDO1 and interferon (IFN)-γ (27, 144, 148, 150, 152–154). Induction of CXCL12, CXCR4 and IDO1 in tumours have been associated with accumulation of myeloid derived suppressor cells (90, 155). Moreover, direct interaction of EP2/EP4 receptors (present on DCs) with the available PGE2 can modulate DC maturation, metalloprotease-driven DC motility, and immune response in tumours (27, 149, 152, 156–158). Thus, targeting the inflammatory environment of the tumour is important to restore DC function to harvest the full potential of immunotherapy.

**LEVERAGING DC BIOLOGY IN CANCER THERAPIES**

Anti-tumour immunity relies on cross-presentation of tumour antigens by DCs to elicit a CD8+ T cell response. Among various DC subsets, cDC1s (XCR1+, CD103+) play a critical role in anti-tumour immunity. CLEC9A, (also known as DNGR1) is highly expressed on cDC1s and binds necrotic cell debris and promotes antigen processing in tumours (159–161). One of the reasons for checkpoint blockade failure is poor antigen presentation due to absence of co-stimulatory molecules and therefore modulation of DC function could increase responses to these therapies. One method to address this issue is the development of DC vaccines for cancer treatment, bypassing the need to activate and mature DCs within the tumour. DC-based cancer vaccines work by recruiting ex-vivo generated dendritic cells (or monocyte derived patient DCs) that are genetically engineered, matured, and loaded with tumour-specific antigens (162–164) or by reprogramming endogenous DCs by injecting biomaterial-based scaffolds providing favourable microenvironment for the recruitment of activated DCs (165, 166). An ideal DC vaccine must be able to increase cross-presentation by DCs, express high levels of co-stimulatory molecules, induce tumour-specific T cells with high migratory and cytolytic capabilities. Furthermore, the use of dendritic growth factor Flt3L in combination with checkpoint inhibitors or DC vaccines has improved number of activated intratumoural cDC1s and enhanced anti-tumour immunity to BRAF and checkpoint blockade in preclinical models (167–170).

Presence of co-inhibitory signals (e.g., IL-10, IL-6, PGE2, TGF-β) or absence of co-stimulatory molecules (e.g. CD80 and CD86) can result in inefficient antigen presentation by DCs and poor induction of antigen-specific CD8+T cells. Therefore, inflammatory cytokines secreted by tumour cells and tumour-associated stroma have been identified as promising candidates to potentiate current immunotherapies including immune checkpoint blockade and CAR-T therapy (149, 171–173). Stroma present around most tumours can also magnify inflammation and impede DC phenotype (174–177) and hence manipulating stroma/DC crosstalk in the TME could help improve DC function.

**DISCUSSION**

It is now established that tumours can exploit their surroundings to create an immunosuppressive microenvironment to control DC function within both the TME and TDLNs (178, 179). These signals including cytokines, chemokines, prostaglandins, growth factors, immune checkpoint genes, etc., may target different DC subsets infiltrating tumours and influence DC maturation, antigen uptake and DC migration (53, 180). Although the success of immunotherapy relies on enhanced T cell activity, activation of tumour-specific T cells cannot be achieved without prior antigen presentation by professional DCs. To overcome immunosuppressive signals, personalized vaccines loaded with patient-derived engineered DCs or delivery of innate stimulus such as TLR3 ligand or a STING agonist to DCs at the tumour site are being developed and have shown promising results (181, 182). Repurposing of existing anti-inflammatory drugs such as aspirin along with DC vaccines or immunotherapies has also been successfully tested in pre-clinical models (149).

This review also addresses the importance of local versus TDLN priming of anti-tumoural T cell responses. Tissue resident memory CD103+ CD8+ T cells residing in the non-lymphoid tissues have shown to provide local immunosurveillance and enhanced immune responses in melanoma, lung and breast tumours (183–187). Moreover, melanoma patients with higher resident T cell population responded better to anti-PD-1 immunotherapy with improved survival (188, 189). However, what is still unclear is how are tissue resident memory CD8+ T cells primed (Figure 2) and whether there is a distinct population of DCs required to activate them. Although the exact regulatory mechanisms remain to be explored further, it is hypothesized that crosstalk between tissue resident memory T cells, tumour cells, stromal cells and DCs within the TME potentiate secondary T-cell responses against tumours (Figure 2). This also opens discussion on the role of tumour associated tertiary lymphoid structures (TLSs) in intra-tumoural DC maturation; and sourcing T cells and B cells to the tumour (190). Although TLS has been positively correlated with anti-tumour responses, there are still many questions remain to be answered such as TLS composition and TLS induction at tumour site before TLS can be adopted as a predictive tool or as a therapeutic option. Our discussion demonstrates the importance of site of antigen presentation in DC maturation and trafficking which must be exploited therapeutically to enhance immune response against cancer.

**AUTHOR CONTRIBUTIONS**

YG and SA planned the concept and design of the review. YG and AK collected previous literature on the topic and drafted the article. YG made the figures. SA performed critical revision of the article. YG and SA edited the final version of the article.

**FUNDING**

This work is funded by Cancer Research UK (Career development fellowship CRUK-A19763 to SA) and Medical Research Council (MC-U12266B).
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Presented to CD8+ T Cells by CD8+ Dendritic Cells. *J Immunol* (2001) 166:5327–30. doi: 10.4049/jimmunol.166.9.5327

38. Schlegel K, McGovern N, Teo P, Zelant A, Atrashki K, Low D, et al. JRF4 Transcription Factor-Dependent CD11b+ Dendritic Cells in Human and Mouse Control Mucosal IL-17 Cytokine Responses. *Immunity* (2013) 38:970–83. doi: 10.1016/j.immuni.2013.04.011

39. Steinman RM. Decisions About Dendritic Cells: Past, Present, and Future. *Annu Rev Immunol* (2012) 30:1–22. doi: 10.1146/annurev-immunol-100311-102839

40. Harjunpää H, Lört Asens M, Guenther C, Fagerholm SC. Cell Adhesion Molecules and Their Roles and Regulation in the Immune and Tumor Microenvironment. *Front Immunol* (2019) 10. doi: 10.3389/fimmu.2019.01078

41. Kobayashi D, Endo M, Ochi H, Hojo H, Miyasaka M, Hayasaki H. Regulation of CCR7-Dependent Cell Migration Through CCR7 Homodimer Formation. *Sci Rep* (2017) 7:1–14. doi: 10.1038/s41598-017-0911-4

42. Morrison VL, James MJ, Grzes K, Cook P, Glass DG, Savinco T. Loss of Beta2-Integrin-Mediated Cytoskeletal Linkage Regrowmend Dendritic Cells to a Mature Migratory Phenotype. *Nat Commun* (2014) 5:1–26. doi: 10.1038/ncomms6359

43. Roberts EW, Broz ML, Binnewies M, Headley MB, Nelson AE, Wolf DM, et al. Critical Role for CD103+/CD141+ Dendritic Cells Bearing CCR7 in the Reviewing of Dendritic Cells in Intestinal Lamina Propria to Mesenteric Lymph Nodes. *J Immunol* (2006) 176:803–10. doi: 10.4049/jimmunol.176.2.803

44. Russo E, Teijeira A, Vahtomaki K, Willrodt A-H, Bloch JS, Nitschke M, et al. Intralymphatic CCL21 Promotes Tissue Egress of Dendritic Cells Through Afferent Lymphatic Vessels. *Cell Rep* (2016) 14:1723–34. doi: 10.1016/j.celrep.2016.01.048

45. Vahtomaki K, Brown M, Hauschuld R, de Vries I, Leithner AF, Mehling M, et al. Locally Triggered Release of the Chemokine CCL21 Promotes Dendritic Cell Transmigration across Lymphatic Endothelia. Cell (2017) 199:902–9. doi: 10.1016/j.celrep.2017.04.027

46. Acton SE, Astariita JL, Malhotra D, Lukacs-Kornek V, Franz B, Hess PR, et al. Podoplanin-Rich Stromal Networks Induce Dendritic Cell Motility via Activation of the C-Type Lectin Receptor CLEC-2. *Immunity* (2012) 37:276–89. doi: 10.1016/j.immuni.2012.05.022

47. Braun A, Worbs T, Moschovakis GL, Guenther C, Fagerholm SC, et al. Afferent Lymph–Derived Dendritic Cells DCs Use Different Chemokine Receptor CCR7-dependent Routes for Entry Into the Lymph Node and Intramural Migration. *Nat Immunol* (2011) 12:879–87. doi: 10.1038/ni.2050

48. Link A, Vogt TK, Fuchs S, Britschgi MR, Dahm-Orbea H, Hinz B, et al. Fibroblastic Reticular Cells in Lymph Nodes Regulate the Homeostasis of Naïve T Cells. *Nat Immunol* (2007) 8:1255–65. doi: 10.1038/ni.1513

49. Peduto L, Dulauroy S, Lochner M, Späth GF, Morales MA, Cumanio A, et al. Inflammation Recaptulates the Ontogeny of Lymphoid Stromal Cells. *J Immunol* (2009) 182:5789–99. doi: 10.4049/jimmunol.0803974

50. Nuñez NG, Tosello Boari J, Ramos RN, Richer W, Cagnard N, Anderfuhren M, et al. Circulating Dendritic Cells. *Nature* (2015) 519:381–8. doi: 10.1038/nature14404

51. Jang MH, Sougawa N, Tanaka T, Hiroi T, Tohya K, et al. CCR7 Is Criticaly Important for Migration of Dendritic Cells in Intestinal Lamina Propria to Mesenteric Lymph Nodes. *J Immunol* (2006) 176:803–10. doi: 10.4049/jimmunol.176.2.803

52. Wang X, Ji J, Zhang F, Fan Z, Zhang L, Shi L, et al. Stimulation of Dendritic Cells by DAMPs in ALA-PDT Treated SCC Tumor Cells. *Oncotarget* (2015) 6:44688–702. doi: 10.18632/oncotarget.9575

53. Bold KF, Schreibelt G, Rabold K, Wecule SK, Schwarze JK, Dziobek A, et al. The Clinical Application of Cancer Immunotherapy Based on Naturally Circulating Dendritic Cells. *J Immunother Cancer* (2019) 7:1–13. doi: 10.1186/s41425-019-0580-6

54. Caronni N, Simoncello F, Stafetta F, Guarinacci C, Ruiz-Moreno JS, Opitz B, et al. Downregulation of Membrane Trafficking Proteins and Lactate Conditioning Determine Loss of Dendritic Cell Function in Lung Inflammation. *Cancer Res* (2018) 78:1685–99. doi: 10.1158/0008-5472.CAN-17-1307

55. Wang X, Ji J, Zhang F, Fan Z, Zhang L, Shi L, et al. Stimulation of Dendritic Cells by DAMPs in ALA-PDT Treated SCC Tumor Cells. *Oncotarget* (2015) 6:44688–702. doi: 10.18632/oncotarget.9575
75. Audiger C, Rahman MJ, Yun TJ, Tarbell K, Lesage S. The Importance of Dendritic Cells in Maintaining Immune Tolerance. *J Immunol* (2017) 198:223–31. doi: 10.4049/jimmunol.1601629

76. del Prete A, Sozio F, Barbazza I, Salvi V, Tiberio L, Lafferanchi M, et al. Functional Role of Dendritic Cell Subsets in Cancer Progression and Clinical Implications. *Int J Mol Sci* (2020) 21:1–24. doi: 10.3390/ijms21113930

77. Scholz F, Grau M, Menzel L, Graband A, Zapukhlyak M, Leutz A, et al. The Transcription Factor C/EBPβ Orchestrates Dendritic Cell Maturation and Functionality Under Homeostatic and Malignant Conditions. *Proc Natl Acad Sci* (2020) 117:26328–39. doi: 10.1073/pnas.2008831117

78. Hernandez A, Burger M, Blomberg BB, Ross WA, Gaynor JJ, Lindner I, et al. Injection of NF-κB During Human Dendritic Cell Differentiation Generates Anergy and Regulatory T-Cell Activity for One But Not Two Human Lekuoyce Antigen DR Mismatches. *Hum Immunol* (2007) 68:715–29. doi: 10.1016/j.jhimm.2007.05.010

79. Aibar S, González-Blas CB, Moerman T, Huynh-Thu VA, Imrichova H, Hulselmann G, et al. SCENIC: Single-Cell Regulatory Network Inference and Clustering. *Nat Methods* (2017) 14:7083–6. doi: 10.1038/nmeth.4463

80. Carena C, Calcaterra F, Orsiolò F, di Vito C, Ubezio M, della Porta MG, et al. Costimulatory Molecules and Immune Checkpoints Are Differentially Expressed on Different Subsets of Dendritic Cells. *Front Immunol* (2019) 10. doi: 10.3389/fimmu.2019.01325

81. Xiao X, Yang G, Bai P, Gui S, Nguyen TMB, Mercado-Uribe I, et al. The Importance of VEGF in the Tumor Microenvironment. *Nat Immunol* (2004) 17:384–45. doi: 10.1038/immunol.173.6.3844

82. Gabrilovich DI, Ishida T, Nadaf S, Ohm JE,Carbonpe . Antibodies to Vascular Endothelial Growth Factor Enhance the Efficacy of Cancer Immunotherapy by Improving Endogenous Dendritic Cell Function. *Clin Cancer Res* : *An Off J Am Assoc Cancer Res* (1999) 5:2963–70.

83. Mashima T, Watsukisu T, Kawata N, Jang M-K, Nagamori A, Yoshida H, et al. Neutralization of the Induced VEGF-A Potentiates the Therapeutic Effect of an Anti-VEGFR2 Antibody on Gastric Cancer. *In Vivo* *Sci Rep* (2011) 1:1–12. doi: 10.1158/0008-5472.CAN-17-1980

84. Brencicova E, Jagger AL, Evans HG, Georgouli M, Laios A, Attard Montalto D, Lemoli RM, et al. Down-Regulates HLA Class II Expression and IL-12 Production of Human Mature Dendritic Cells Through the VEGF Receptor 2-RhoA- Gi2/ki4 Effect. *Cancer Biol Ther* (2017) 18:5214–20. doi: 10.1089/cmb.2017.00978

85. Rhode PR, Egan JO, Xu W, Hong H, Webb GM, Chen X, et al. Comparison of the Superagonist Complex, ALT-803, to IL15 as Cancer Immunotherapeutics in Animal Models. *Cancer Immunol Res* (2016) 4:49–60. doi: 10.1158/2326-6066.CIR-15-0093-T

86. Barry KC, Hsu J, Broz ML, Cueto FJ, Parone CA, Minha C, et al. Natural Killer–Dendritic Cell Axis Determines Checkpoint Therapy–Responsive Tumor Microenvironments. *Nat Med* (2018) 24:1178–91. doi: 10.1038/s41591-018-0085-8

87. Bai W, Zhang W, Hu B. Vascular Endothelial Growth Factor Suppresses Dendritic Cells Functions of Human Prostate Cancer. *OncoTargets Ther* (2018) 11:627–74. doi: 10.2147/OTT.S161302

88. Cheng J, Deng Y, Hu Y, Wang G, Fu B, Chen W, et al. Hepatic Carcinoma-Associated Fibroblasts Induce IDO-Producing Regulatory Dendritic Cells with Enhanced Inhibition of the Antigen-Specific CD8 T Cell Response. *Sci Rep* (2017) 7:10564.doi: 10.1038/s41598-017-19060-x

89. Ohno Y, Kitamura H, Takahashi N, Ohtake J, Kaneumi S, Sumida K, et al. IL-6 Expression De Drives Immune Evasion in Genetically Altered Mouse. *J Immunol Res* (2018) 2018:175. doi: 10.1155/2018/175. doi: 10.1038/41591-018-0085-8

90. Trabanelli S, Lecciso M, Salvestrini V, Cavo M, Ocadillova D, Lemoli RM, et al. PGE2 -Inuced IDO1 Inhibits the Capacity of Fully Mature DCs to Elicit an *In Vitro* Antileukemic Immune Response. *J Immunol Res* (2015) 2015:1–10. doi: 10.1155/2015/253191

91. Xu X, Liu X, Long J, Hu Z, Zheng Q, Zhang C, et al. Interleukin-10 Reorganizes the Cytoskeleton of Mature Dendritic Cells Leading to Their Impaired Biophysical Properties and Motilities. *PloS One* (2017) 12:1–15. doi: 10.1371/journal.pone.0172523

92. Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O’Shea KS, et al. Heterozygous Embryonic Lethality Induced by Targeted Inactivation of the VEGF Gene. *Nature* (1996) 380:439–42. doi: 10.1038/380439a0
Grasso CS, Tsai J, Onyshchenko M, Abrii-Rodriguez G, Ross-Macdonald P, Wind-Rotolo M, et al. Conserved Interferon-γ Signaling Drives Clinical Response to Immune Checkpoint Blockade Therapy in Melanoma. Cancer Cell (2020) 38:500–15. doi: 10.1016/j.ccell.2020.08.005

Polly VS, Moeini A, Roeflofen LM, Bonavita E, Bell CR, Hutton C, et al. Adaptive Cellular Therapy With T Cells Expressing the Dendritic Cell Growth Factor Flt3L Drives Epitope Spreading and Antitumor Immunity. Nat Immunol (2020) 21:914–26. doi: 10.1038/s41590-020-0676-7

Salmon H, Idoyaga J, Rahman A, Leboeuf M, Remark R, Jordan S, et al. Expansion and Activation of CD103 + Dendritic Cell Progenitors at the Tumor Site Enhances Tumor Responses to Therapeutic PD-L1 and BRAF Inhibition. Immunity (2016) 44:924–38. doi: 10.1016/j.immuni.2016.03.012

Hou J, Karin M, Sun B. Targeting Cancer-Promoting Inflammation — Have Anti-Inflammatory Therapies Come of Age? Nat Rev Clin Oncol (2021) 18:99–115. doi: 10.1038/s41573-018-0044-8

Kobayashi H, Enomoto A, Woods SL, Burt AD, Takahashi M, Worthley DL. Cancer-Associated Fibroblasts in Gastrointestinal Cancer. Nat Gastroenterol Hepatol (2019) 16:282–95. doi: 10.1038/s41575-019-0115-0

Liu T, Han C, Wang S, Fang P, Ma Z, Xu L, et al. Cancer-Associated Fibroblasts: An Emerging Target of Anti-Cancer Immunotherapy. J Hematol Oncol (2019). doi: 10.1186/s13045-019-0770-1

Ziani L, Chouaib S, Thery I. Alteration of the Antitumor Immune Response by Cancer-Associated Fibroblasts. Front Immunol (2018) 9:414. doi: 10.3389/fimmu.2018.00414

Wang J-B, Huang X, Li F-R. Impaired Dendritic Cell Functions in Lung Cancer: A Review of Recent Advances and Future Perspectives. Cancer Immunol Immunother (2019) 68:1–9. doi: 10.1007/s00262-018-2044-3

Wculek SK, Cueto FJ, Mujal AM, Melero I, Krummel MF, Sancho D. Dendritic Cells in Cancer Immunology and Immunotherapy. Nat Rev Cancer (2020) 20(1):7. doi: 10.1038/s41561-019-0180-9

Martin-Fontecha A, Sebastiani S, Höpken UE, Ugovicz M, Lipp M, Lanzavecchia A, et al. Regulation of Dendritic Cell Migration to the Draining Lymph Node. J Exp Med (2003) 198:615–21. doi: 10.1084/jem.20030448

Perez CR, de Palma M. Engineering Dendritic Cell Vaccines to Improve Cancer Immunotherapy. Nat Commun (2019) 10:1–10. doi: 10.1038/s41467-019-13368-y

Ramanjulu JM, Pesiridis GS, Yang J, Concha N, Singh R, Zang S-Y, et al. Design of Amidoindenzoimidazole STING Agonists With Systemic Activity. Nature (2018) 564:439–43. doi: 10.1038/s41586-018-0705-y

Ganesan A-P, Clarke J, Wood O, Garrido-Martin EM, Chee SJ, Mellows T, et al. Tissue-Resident Memory Features Are Linked to the Magnitude of Cytotoxic T Cell Responses in Human Lung Cancer. Nat Immunol (2017) 18:940–50. doi: 10.1038/ni.3775

Malik BT, Byrne KT, Vella JL, Zhang P, Shabaneh TR, Steinberg SM, et al. Resident Memory T Cells in the Skin Mediate Durable Immunity to Melanoma. Sci Immunol (2017) 2:1–24. doi: 10.1126/sciimmunol.aam6346

Park SL, Buzzi A, Rutela J, Hor JL, Hochheiser K, Efferen M, et al. Tissue-Resident Memory CD8+ T Cells Promote Melanoma–Immune Cell Cytotoxic Activity. J Immunother (2006) 29:545–57. doi: 10.1016/j.jimmunol.2007.01.017

Palucka AK, Ueno H, Connolly J, Kerneis-Norvell F, Blanck J-P, Johnston DA, et al. Dendritic Cells Loaded With Killed Allogeneic Melanoma Cells Can Induce Objective Clinical Responses and MART-1 Specific CD8+ T-Cell Immunity. J Immunother (2006) 29:545–57. doi: 10.1016/j.jimmunol.2007.01.017

Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K, et al. Vaccination With Irradiated Tumor Cells Engineered to Secretive Murine Granulocyte-Macrophage Colony-Stimulating Factor Stimulates Potent, Specific, and Long-Lasting Anti-Tumor Immunity. Proc Natl Acad Sci (1993) 90:3539–43. doi: 10.1073/pnas.90.8.3539

Mach N, Dranoff G. Cytokine-Adjuvant Tumor Cell Vaccines. Curr Opin Immunol (2000) 12:571–5. doi: 10.1016/S0952-7915(00)00144-8

Bhardwaj N, Friedlander PA, Pavlick AC, Ernstoff MS, Gastman BR, Hanks BA, et al. Flt3 Ligand Augments Immune Responses to Anti-DE-205-NY-ESO-1 Vaccine Through Expansion of Dendritic Cell Subsets. Nat Cancer (2020) 1:1204–17. doi: 10.1038/s41598-020-00413-y

Hammerich L, Marron TU, Upadhayay R, Svensson-Arvelund J, Dhmaint N, Hussein S, et al. Systemic Clinical Tumor Regressions and Potentiation of PD1 Blockade With in Situ Vaccination. Nat Med (2019) 25:814–24. doi: 10.1038/s41591-019-0410-x

Lai J, Mardiana S, House IG, Sek K, Henderson MA, Giufrida L, et al. Adaptive Cellular Therapy With T Cells Expressing the Dendritic Cell Growth Factor Flt3L Drives Epitope Spreading and Antitumor Immunity. Nat Immunol (2020) 21:914–26. doi: 10.1038/s41590-020-0676-7
Equilibrium in Skin. Nature (2019) 565:366–71. doi: 10.1038/s41586-018-0812-9

Savas P, Virassamy B, Ye C, Salim A, Mintoff CP, Caramia F, et al. Single-Cell Profiling of Breast Cancer T Cells Reveals a Tissue-Resident Memory Subset Associated With Improved Prognosis. Nat Med (2018) 24:986–93. doi: 10.1038/s41591-018-0078-7

Schenkel JM, Fraser KA, Beura LK, Pauken KE, Vezys V, Masopust D. Resident Memory CD8 T Cells Trigger Protective Innate and Adaptive Immune Responses. Science (2014) 346:98–101. doi: 10.1126/science.1254536

Edwards J, Wilmott JS, Madore J, Gide TN, Quek C, Tasker A, et al. CD103⁺ Tumor-Resident CD8⁺ T Cells Are Associated With Improved Survival in Immunotherapy-NAïve Melanoma Patients and Expand Significantly During Anti–PD-1 Treatment. Clin Cancer Res (2018) 24:3036–45. doi: 10.1158/1078-0432.CCR-17-2257

León-letelier RA, Castro-Medina DI, Badillo-Godínez O, Tepale-Segura A, Huanosta-Murillo E, Aguilar-Flores C, et al. Induction of Progenitor Exhausted Tissue-Resident Memory CD8⁺ T Cells Upon Salmonella Typhi Porins Adjuvant Immunization Correlates With Melanoma Control and Anti-PD-1 Immunotherapy Cooperation. Front Immunol (2020) 11:583382. doi: 10.3389/fimmu.2020.583382

Sautès-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary Lymphoid Structures in the Era of Cancer Immunotherapy. Nat Rev Cancer (2019) 19:1–21. doi: 10.1038/s41568-019-0144-6

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