Experimental Hematology & Oncology

REVIEW Open Access

Tumor battlefield within inflamed, excluded or desert immune phenotypes: the mechanisms and strategies

Siwei Zheng[1,2†], Wenwen Wang[3†], Lesang Shen[1,2], Yao Yao[1,2], Wenjie Xia[4*] and Chao Ni[1,2*]

Abstract

The tumor microenvironment demonstrates great immunophenotypic heterogeneity, which has been leveraged in traditional immune-hot/cold tumor categorization based on the abundance of intra-tumoral immune cells. By incorporating the spatial immune contexture, the tumor immunophenotype was further elaborated into immune-inflamed, immune-excluded, and immune-desert. However, the mechanisms underlying these different immune phenotypes are yet to be comprehensively elucidated. In this review, we discuss how tumor cells and the tumor microenvironment interact collectively to shape the immune landscape from the perspectives of tumor cells, immune cells, the extracellular matrix, and cancer metabolism, and we summarize potential therapeutic options according to distinct immunophenotypes for personalized precision medicine.

Keywords Tumor microenvironment, Tumor immune phenotype, Immunotherapy, Tumor metabolism

Background

The past decades have witnessed encouraging practices in immunotherapy that have revolutionized the field of oncology by highlighting the host immune response as a viable target for cancer treatment. The most prospective approaches are immune checkpoint blockade (ICB) and adoptive cell therapy (ACT), as these strategies focus on key fighters in the anti-tumor battle [1]. ACT has shown remarkable achievements with six FDA-approved chimeric antigen receptor (CAR)-T cell therapies, particularly in hematologic malignancies in which a well-defined spatial structure is absent [2]. ICB primarily includes inhibitors of programmed cell death 1 (PD-1), programmed cell death ligand 1 (PD-L1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) [3]. As a monotherapy, ICB can yield beneficial and long-term therapeutic responses in patients with various types of cancer, with response rates ranging from 10 to 58% [4–6]. The mechanisms underlying such different likelihoods of response remain elusive and may be ascribed to the spatial heterogeneity of the preexisting tumor immune microenvironment (TIME) [7].

The TIME is composed of neoplastic cancer cells and non-cancer components, including multiple stromal and immune cells, vascular system, the extracellular matrix (ECM) compartments. Cancer cells actively orchestrate

†Siwei Zheng and Wenwen Wang contributed equally to this work.

*Correspondence:
Wenjie Xia
xiawenjie1031@zju.edu.cn
Chao Ni
drnichao@zju.edu.cn
[1]Department of Breast Surgery, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310000, China
[2]Key Laboratory of Tumor Microenvironment and Immune Therapy of Zhejiang Province, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310000, China
[3]Department of Pathology, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310000, China
[4]General Surgery, Cancer Center, Department of Breast Surgery, Zhejiang Provincial People's Hospital (Affiliated People's Hospital, Hangzhou Medical College), Hangzhou 310000, China

a tumor-supportive microenvironment, whereas the evolving TIME selects certain tumor subclones with survival advantages [8, 9]. This complex interplay is partly reflected in the immune landscape, which is considered the focal point of the TIME [10–14]. Traditionally, it is believed that the number of tumor-infiltrating lymphocytes (TILs) serves as a predictor for immunotherapy susceptibility and prognosis; therefore, tumors are dichotomized into immune-hot (abundant infiltration of $CD8^+$ T cells) and immune-cold (limited infiltration of $CD8^+$ T cells) phenotypes [15–17]. The Immunoscore is a worldwide accepted and standardized scoring system for colorectal cancer (CRC) that quantifies the density of $CD3^+$ and $CD8^+$ T cells within the tumor center and invasive margin. By introducing immune parameters, the Immunoscore has been validated to outperform other prognostic indicators, including pathologic T and N stages, lymphovascular invasion, tumor differentiation, and microsatellite instability (MSI) status [18, 19]. Nonetheless, the GeparNeuvo trial (NCT02685059), which was stratified by the quantity of stromal tumor-infiltrating lymphocytes (sTILs) before neoadjuvant chemotherapy, showed that sTIL status is not statistically significant in predicting invasive disease-free survival and pathological complete response, emphasizing the need for comprehensive knowledge of distinct spatial patterns of the TIME [20].

A decade before, Chen and Mellman proposed a novel trichotomic classification of tumor immunophenotypes. Based primarily on the spatial and quantity distribution of immune cells ($CD8^+$ T cells in particular) within tumor nest or stromal compartments, the TIME can be morphologically defined into "immune-inflamed", "immune-excluded" and "immune-desert" with distinctive traits [13, 21, 22]. The Impassion130 (NCT02425891) trial revealed a declining tendency of PD-L1 expression in tumor cells following the aforementioned order, indicating a significant difference in immunotherapy susceptibility across the three immunophenotypes [23]. The immune-excluded phenotype, newly identified from the traditional "hot/cold" classification, has been unveiled to be associated with ICB resistance [24]. One plausible explanation for this phenomenon is that activated $CD8^+$ T cells are excluded from the tumor parenchyma and, therefore, cannot effectively kill malignant cells owing to their limited ability to penetrate the tumor core. However, a comprehensive knowledge of the factors contributing to different immunophenotypes remains to be elucidated. In this review, we summarize the interrelationships between tumor cells and the TIME that underlie distinctive immunophenotypes. The ultimate goal was to identify the biologicals vulnerabilities of cancer and provide a rationale for precise anti-tumor treatments.

Immune features of the Inflamed, excluded and Desert TIME (Fig. 1)

Conventionally, immune-inflamed tumors (so-called "hot" or "immune-infiltrated" tumors) are characterized by a profusion of TILs both in the tumor nests and stroma. Patients with inflamed tumors, which account for up to 50% of all human tumors, generally portend favorable response towards chemotherapy and ICB [21, 25–27]. The immune-inflamed phenotype is associated with elevated genomic instability and antigenicity [21], along with an accumulation of proinflammatory cytokines and an increased interferon response; however, whether the inflammatory TIME is the cause or consequence of immune cell influx remains an open question [25].

Immune-desert (also referred to as "cold" or "ignored") tumors, as the name suggests, indicate a paucity or absence of T cells either in the tumor core or periphery, though myeloid cells may be present instead [21, 25, 28, 29]. This immunophenotype features defective antigen presentation machinery (APM) and exhibits a reduced interferon (IFN) response, as well as an expansion of immunosuppressive cells [21, 30–32]. As reported, chemotherapy or ICB treatment remains dismal towards immune-desert tumors.

Finally, immune-excluded tumors show a distinctive T-cell immune context [25]. $CD8^+$ T cells are located in the vicinity of the tumor parenchyma but are incapable of penetrating and having direct dialogue with tumor cells. Instead, immune cells circumferentially "stuck" in the peritumoral fibroblast- and collagen-rich stroma [21, 29, 33, 34]. This implies ineffective T cell activation, proliferation, and trafficking [25]. Given its failure to mount an efficient immune response, the efficacy towards ICB is generally inferior to that of immune-inflamed tumors, although some indicate an even worse prognosis than the desert immunophenotype, which is of significant clinical relevance [35–37]. In an analysis of the ICOL7 trial ovarian cancer cohort, patients with immune-excluded phenotypes had shorter progression-free survival than those with either inflamed or desert phenotypes [38]. The biological mechanisms underlying the immune-excluded phenotype remain inconclusive [28, 38] (Fig. 1).

The phenotypic classification of the TIME is complicated by inter- and intra-tumoral heterogeneity, which appeals for a clear-cut consensus to drive further applications (Fig. 2) [39, 40]. Currently, Immunohistochemistry (IHC) is the most commonly used technique for evaluating immune infiltration patterns as it allows for the quantification of immune cells in terms of their type, density, and distribution [37]. However, an important question is whether human tissues, which are intrinsically three-dimensional (3D), are examined as limited two-dimensional cross-sections that may potentially misrepresent the entire tumor landscape due to sampling bias [41].

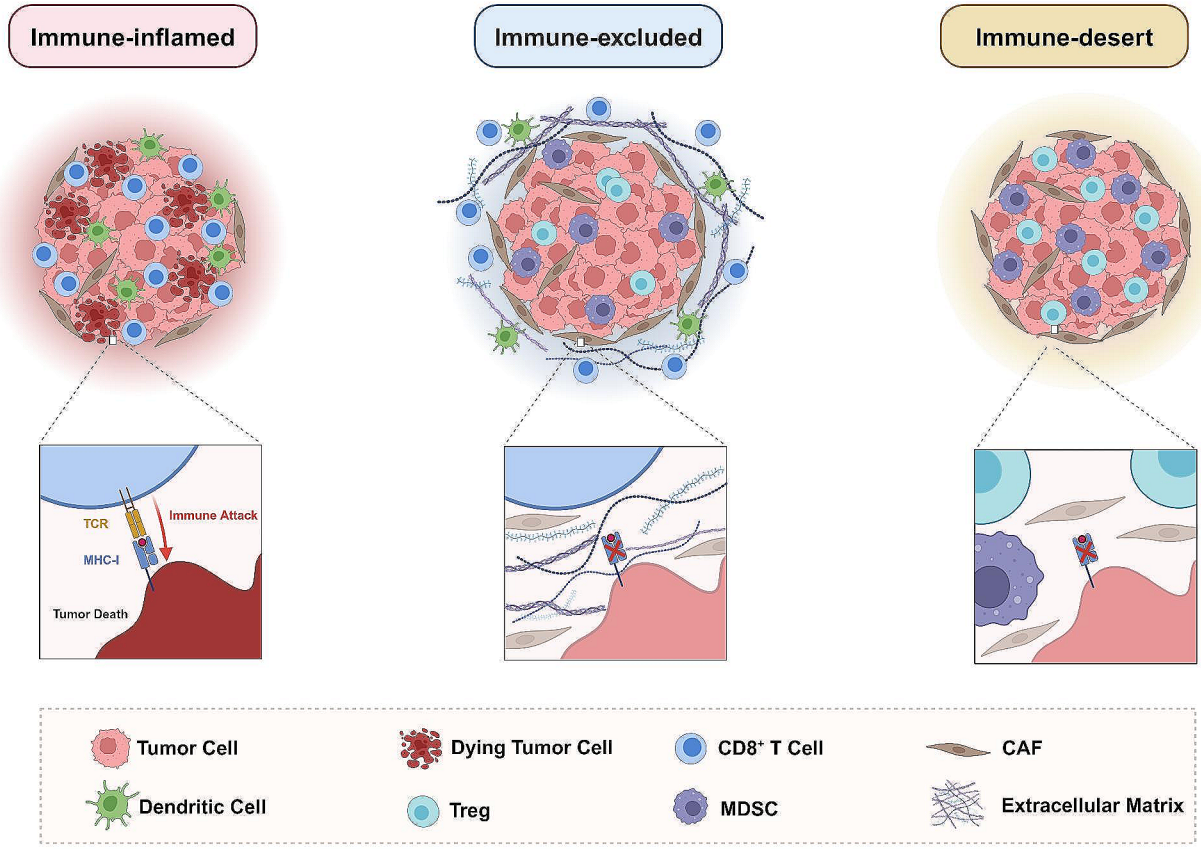

Fig. 1 The Schematic Diagram of Immune-inflamed, Immune-excluded, and Immune-desert Tumors. The immune-inflamed tumors are characterized by an abundant infiltration of $CD8^+$ T cells within the tumor parenchyma, which involves active tumoricidal immune attacks. While for the immune-excluded tumors, $CD8^+$ cells are generally trapped in the peritumoral stroma, failing to directly eliminate tumor cells. As for the immune-desert tumors, $CD8^+$ T cells are barely present either in the parenchymal or stromal sites, instead, immune suppressive cells (e.g. Tregs, MDSCs) may abound in the TIME

Technical constraints have hindered the holistic characterization of TIME, as a larger tissue volume mitigates sampling bias and accounts for tissue heterogeneity [42]. Recently, a 3D pathology deep learning platform, TriPath, has demonstrated superior prognostic performance over traditional two-dimensional slice-based approaches, indicating its potential clinical applications in defining tumor immunophenotypes [41]. Furthermore, immune monitoring challenges persist, as immune parameters are dynamically altered during tumor progression [43]. One viable diagnostic procedure, immunopositron-emission tomography imaging, holds great promise for the non-invasive tracking of intra-tumoral $CD8^+$ T cells [44]. In addition, other authors have constructed gene signature-based categorizations using transcriptome analysis [45, 46]. For instance, genes enrichment of IFN-γ pathway are usually recognized as "inflamed" tumors and signatures of stromal biology are indicative for immune-excluded ones, while absence of both is categorized as immune-desert [33, 47–49]. Several techniques based on the deconvolution of bulk gene expression data have been developed to predict the level of intra-tumoral immune infiltrates, including CIBERSORT (which estimates the proportional distribution of immune subsets within the overall leukocyte population) [46, 50]; xCell (which evaluates the abundance of immune cells within the TIME) [51]; TIMER (which calculates enrichment scores by analyzing the proportions of immune and stromal cell types) [52] and integrated immunogenomics methods that can be employed to identify immune subtypes of cancer with a CIBERSORT-based approach [48]. Nevertheless, these immunophenotype-predicting techniques have inevitable limitations in terms of inconsistency during the RNA extraction procedure, the impossibility of univocally assigning transcripts to specific cell subsets, and discrepancies in immunophenotypes between circulating blood and the TIME across cancer types. However, the high cost of novel single-cell based approaches [53] and in situ barcode sequencing [54] hinders their large-scale diagnostic applications.

Notably, these immunophenotype classification methods are not simply one-size-fits-all methods; some

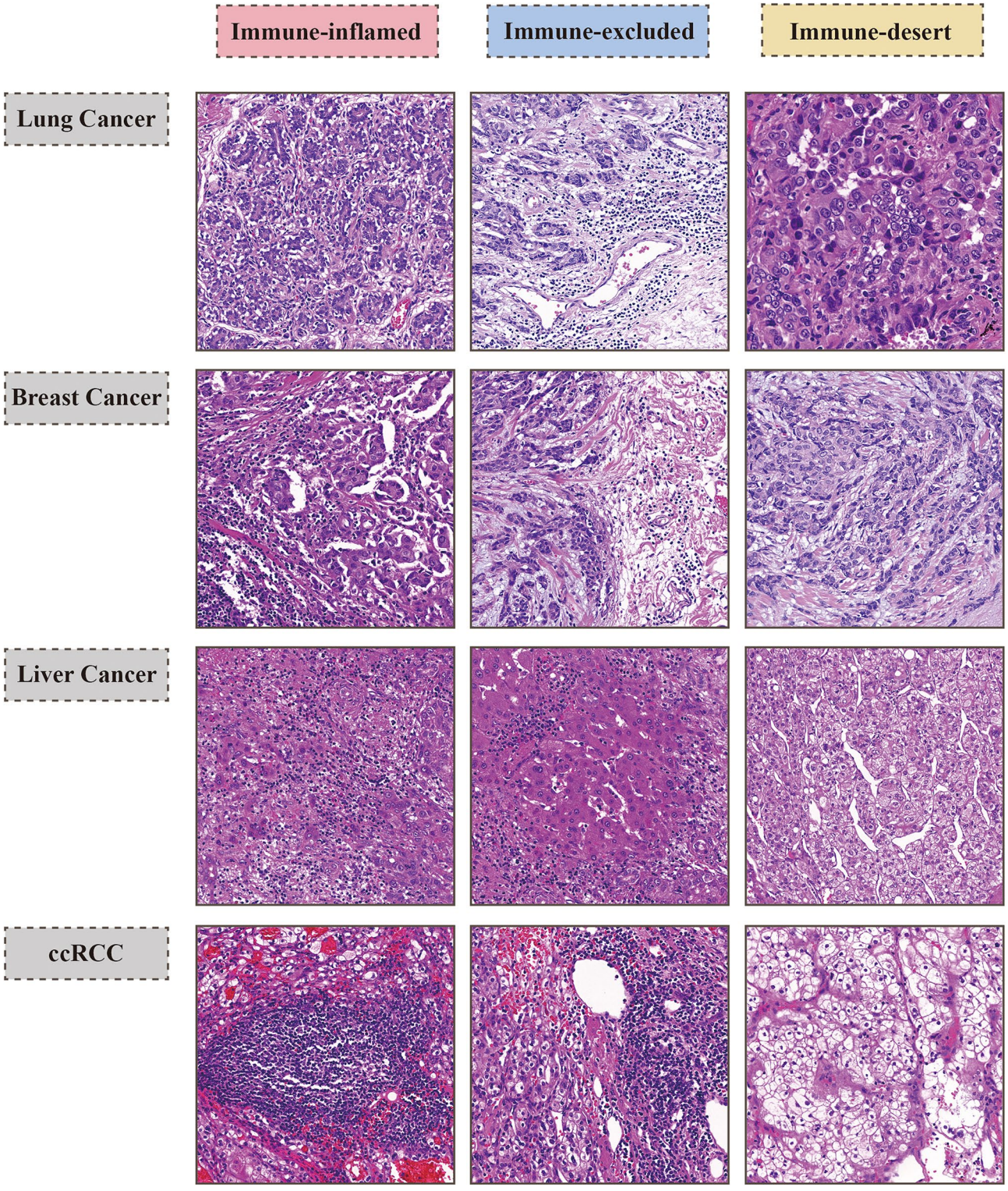

Fig. 2 Representative HE Images of Corresponding Tumor Immune Phenotypes from Lung Cancer, Breast Cancer, Liver Cancer, and Clear Cell Renal Cell Carcinoma

tumors possess attributes that span multiple categories [55]. Desbois et al. have highlighted that the quantity and landscape of immune cells within the TIME are continuous [38]. In other words, the immunophenotypes of specific tumor types differ among individuals and should be comprehensively evaluated on a case-by-case basis (Table 1). CRCs are primarily composed of the immune-excluded subtype with up to 70–75% frequencies, while only 10% of cases exhibit an inflamed TIME. Conversely, non-small cell lung cancers (NSCLC) show approximately 40% excluded and 30–35% inflamed phenotype [13]. Breast cancer (BC) is histologically purported as a "cold" tumor type while in-depth research has uncovered its strong immunophenotypic heterogeneity among subtypes [56, 57]. Basal-like subtype represents the largest fraction of the "inflamed" BC, followed by HER-2 and Luminal-B tumors. Triple negative breast cancer (TNBC) was typically considered as the most immunogenic subtype, however, approximately 28% and 26% of TNBC cases exhibit an immune-desert and immune-excluded pattern respectively, clearly contradicting the notion that TNBC is "inflamed" [58]. Moreover, the metastasized TNBC exhibits a divergent pattern from primary lesions, consisting of higher proportions of the excluded (41%) and desert (37%) phenotypes [58]. For now though, due to the great heterogeneity across solid tumors, as well as a lack of reliable biomarkers, the definitions of inflamed, excluded and desert tumor types are far from consistent. An increasing number of studies have explored the mechanisms underlying various TIME subtypes during tumorigenesis. Here, we comprehensively review the pertinent literatures that is expected to improve treatment strategies based on tumor immunophenotypes for tailored-comers in the future.

Table 1 Proportions of immune-inflamed, immune-excluded, and immune desert subtypes from different tumors

Cancer Type	Immune-inflamed	Immune-excluded	Immune-desert	Reference
TNBC	46%	26%	28%	https://doi.org/10.1038/s41467-021-25962-0
NSCLC	35%	40%	25%	https://doi.org/10.1016/j.immuni.2019.12.011
Pancreatic Cancer	44%	46%	10%	https://doi.org/10.3390/ijms25010142
CRC	10%	75%	15%	https://doi.org/10.1016/j.immuni.2019.12.011
Ovarian Cancer	27%	45%	28%	https://doi.org/10.3390/cancers14174246
mUC	26%	47%	27%	https://doi.org/10.1038/nature25501
mTNBC	22%	41%	37%	https://doi.org/10.1038/s41467-021-25962-0
HCC	31%	24%	45%	https://doi.org/10.7150/jca.54408
ccRCC	22%	19%	59%	https://doi.org/10.1038/s41379-021-00864-0

Abbreviations: TNBC: Triple Negative Breast Cancer; NSCLC: Non-small Cell Lung Cancer; CRC: Colorectal Cancer; mUC: metastatic Urothelial Cancer; mTNBC: metastatic Triple Negative Breast Cancer; HCC: Hepatocellular Carcinoma; ccRCC: clear cell Renal Cell Carcinoma

The mechanisms of anti-tumor immune anergy in different TIME

Tumor antigen recognition initiates the cancer-immunity cycle, triggering cascades that involve antigen presentation, immune activation, cytotoxic effector cell trafficking, infiltration into the tumor nest, and the recognition and destruction of cancer cells [25, 59, 60]. Lynch syndrome (LS) is a hereditary CRC syndrome caused by germline mutations in the DNA repairing machinery. The consequent excessive mutational load and neoantigen levels contribute to the enrichment of TILs within the TIME, resulting in a striking immune inflammation [61]. Despite the adaptive immune system, physical barriers may pose hurdles to the direct elimination of cancer. Fibrous tissues and stromal components encase the tumor tightly, whereas internal tumor endothelial cells may exhibit a dysfunctional morphology and phenotype, rendering them unresponsive to inflammatory signals. In this context, $CD8^+$ T-cell trafficking and infiltration into tumor nests are obstructed. Overall, the status of the TIME could be modulated by any step in the cancer immunity cycle, which results in a specific tumor immunophenotype and implies available therapeutic targets.

Genomic instability and TILs heterogeneity

Genomic instability (GI) drives tumor evolution, which is conceptually measured by tumor mutational burden (TMB) and MSI [62]. The U.S. food and drug association has proposed high tumor mutational burden (TMB-H) at a cutoff value of ≥10 mut/Mb, which serves as a prognostic predictor of survival rates and ICB responses for solid tumor patients [63]. This suggests a positive correlation between genomic alterations and higher immunogenicity, potentiating cancer-responsive TILs [64–68]. Immune-inflamed tumors typically present with a high

TMB status or deficiencies in DNA repair mechanisms, such as mismatch repair deficiency, and subsequent MSI. These characteristics increase the neoantigen load and attract TILs to the tumor parenchyma [69–71]. Studies on various hypermutated malignancies, including melanoma and lung, bladder, and urothelial cancers, have provided compelling evidence of an inflamed immune microenvironment [33, 64, 72]. However, somatic copy number alterations (SCNAs), a form of GI, have adverse effects [48]. A comprehensive bioinformatics analysis by Davoli et al. recapitulated compromised cytotoxic activities and CTL infiltration in tumors with high SCNA levels, demonstrating reduced expression of genes encoding components of the T-cell receptor (TCR) complex, as well as genes mediating cytotoxic functions and a proinflammatory TIME [73]. From a therapeutic standpoint, combining the tumor SCNA score with TMB has proven to be a more effective survival predictor for patients receiving immunotherapy than using either biomarker alone. In addition to GI, exogenous carcinogens such as viral infections are implicated in both tumorigenesis and progression by integrating viral genomes into the host [74, 75]. Human endogenous retroviruses are prevalent in malignancies like clear cell renal cell carcinoma (ccRCC) and ovarian cancer, which exhibit upregulated IFN-γ signatures and immune-inflammation [76, 77]. In contrast, non-inflamed tumors are generally characterized by stable genomes and low immunogenicity [13, 78]. The relationship between GI and tumor immunophenotypes is intricate and possibly contingent on a specific tumor type. Gastroesophageal adenocarcinoma (GEA) is a highly heterogenous cancer, and subtypes with MSI or Epstein-Barr Virus positivity demonstrate intense T-cell infiltrates with robust ICB efficacy [79]. However, most chromosomally unstable GEAs are associated with the immune-exclusion phenotype. The diffuse/genome-stable GEA group exhibited enrichment of $CD4^+$ T cells rather than cytotoxic $CD8^+$ T cells, but the mechanisms are still unknown.

Mutations of the tumor-intrinsic pathways may also indicate a specific immune cell context. For instance, the STING pathway has been linked to the immune-inflamed phenotype as it triggers type I interferons and key chemoattractant for T cell trafficking [80]. A recent report on gastric cancer demonstrated that how human epidermal growth factor receptor-2 (HER2) heterogeneity complicated the TIME is essentially regulated by the STING signaling pathway, whereas HER2-high areas remain immunologically inactivated [81]. Novel cocktail strategies combing the STING agonist bivalent manganese or MSA-2 with anti-TGF-β/PD-L1 bispecific antibody YM101 has successfully inflamed immune-desert tumors, while YM101 alone is insufficient to achieve this outcome [59, 82, 83]. In a T-cell-inflamed mouse model of head and neck squamous cell carcinoma, local injection of a STING agonist into the tumor lesion, followed by anti-PD-L1 treatment, led to successful tumor control and complete rejection [84]. STAT3, an integral signaling node in various oncogenic pathways, is constitutively activated in several malignancies, including lung, breast, and liver cancers. In collaboration with hypoxia-inducible factor-1 (HIF-1), STAT3 orchestrates TWIST1 expression, a crucial marker of epithelial-mesenchymal transition [85]. In immunologically inactive BC, it has been observed that the downregulation of TGF-β in stromal fibroblasts can lead to an upregulation of CXCL1, which then activates STAT3 by acting on CXCR2 in tumor cells [86]. In addition, abnormal activation of WNT/β-catenin pathway has been extensively observed in tumors with high TMB but limited T cell signatures, demonstrating CTL exclusion and blunted immune responses [13, 87–90]. Mechanistically, the overexpression of β-catenin interrupts IFN-γ production in an IL-10-independent manner, as well as reduces the chemoattractant for $CD103^+$ dendritic cells (DCs), thereby leading to impaired activation of cytotoxic cells [91]. Moreover, dysregulated WNT/β-catenin signaling is a significant factor in the self-renewal and differentiation of cancer stem cells in various solid tumors [92]. Furthermore, PTEN deletion/PI3K activation have been observed in immune-excluded tumors, leading to resistance towards ICB therapy in both mouse models and human melanoma cases [93]. Promisingly, evidence suggested that a PI3Kβ inhibitor could synergistically enhance tumor control in vivo, holding out hope for leveraging these immune-exclusionary oncogene pathways to bolster immunotherapy efficacy [94].

High levels of GI does not necessarily equate to an immune-inflamed TIME, as TILs are of functional and phenotypic diversity, among which effector $CD8^+$ T cells primarily favor the inflamed immunophenotype [95]. Interestingly, Yang et al. have recently identified two patterns of immune "cold", namely "quantitative cold" and "qualitative cold" in primitive and omental metastatic lesions of ovarian cancer, respectively [96]. The "quantitative cold" TIME is characterized by a high proportion of immunosuppressive regulatory T cells (Tregs) and limited infiltrated $CD8^+$ T cells, many of which undergo "exhaustion" due to chronic antigen stimulation within the local ovarian ecosystem [97, 98]. The proportion of Tregs within tumors often exceeds 50% of all T cells, nearly ten times the homeostatic frequency in normal blood and lymphoid tissues [99]. Although Tregs and CTLs can both be recruited to the TIME, Tregs have the potential to impede the further infiltration of their cytotoxic counterparts [100]. Indeed, the ratio of intratumoral CTLs to Tregs has been identified as a predictor for immunophenotyping, with higher values often

observed in immune-inflamed tumors [101]. The murine model of EMT6 BC is a typical example of immune-excluded phenotype, recent studies indicate that TGF-β supports the dominance of T progenitor-exhausted cells in the intra-tumoral T-cell pool. As the name suggests, the T progenitor-exhausted cells are the originate of exhausted T cells, which are characterized by a progressive loss of effector functions and elevated levels of co-inhibitory receptors, such as PD-1. Previous research by Castiglioni et al. revealed the dual blockade of PD-L1 and TGF-β, along with the ensued IFN-γ signaling activation, yielded an inflamed TIME [26]. In terms of mechanism, the dual targeting strategy facilitated a higher number of functional stem-cell like $CD8^+$ T cells to develop along effector differentiation trajectory and the intra-tumoral accumulation of $IFN\gamma^{hi}$ $CD8^+$ T cells triggered TIME-wide IFN licensing, which prompted APM as well as enhanced production of T cell stimulatory cytokines and chemokines [21]. Moreover, $CD4^+$ T helper cells can contribute to the reversal of $CD8^+$ T cell exhaustion. In an immune-desert murine model of B16-F10 melanoma, Zander et al. revealed that $CD4^+$ T cell-derived IL-21 can reprogram $CD8^+$ T cells and drive their differentiation into protective cytotoxic CX_3CR1^+ $CD8^+$ T cells, resulting in a more than two-fold increase in their proportion within the TIME [102]. In contrast, the omental metastatic lesions of ovarian cancer have a preponderance of "bystander" T cells that are only responsive to tumor-irrelevant antigens, which are incapable to initiate tumor-specific immune responses [96]. As indicated by TCR repertoire profiling in ovarian and colorectal cancers, only a minor fraction of $CD8^+$ TILs are tumor-specific, while the majority consists of $CD39^-$ $CD8^+$ "bystander" T cells [97, 103, 104]. Interestingly, "bystander" T cells are exempt from the "exhaustion" program and retain functional memory properties, as they remain ignorant of tumor cells [105]. Both preclinical and clinical investigations have evidenced that "bystander" T cells exhibit low expression level of checkpoint receptors [106]. To leverage the distinctive traits of "bystander" T cells, Chen et al. have developed an engineered oncolytic virus to redirect the antigen specificity of malignant cells to the pre-existing "bystander" T cells, which could synergize with PD-1 and/or PD-L1 ICB therapies for immune-inflamed tumors while sensitize "cold" tumors [107]. These discoveries emphasize that the quality of infiltrates holds equal significance to their quantity, as some TIL-low cancers may have diversified TCR clonality [108, 109]. Functional testing of TCRs has revealed pancreatic ductal adenocarcinoma (PDAC), a typically immune-desert tumor, and high frequencies of tumor-reactive (TR) TCR clonotypes in certain genomically unstable samples. PDAC cases with germline mutations in DNA damage repair genes (such as BRCA1, PALB2) identified TR TCRs, whereas genetically stable samples were mostly dominated by bystander TCR clones [110]. The highest TCR-Vβ diversity, as well as the most skewed TCR-Vβ repertoire (harboring clonally expanded reads) have been observed in the inflamed phenotype, enabling to recognize a wide range of cancerous mutations. Conversely, both of these parameters were generally low in the exclusion and desert phenotypes [58]. Notably, the WNT pathway inversely correlates with the skewing of the TCR repertoire, whereas immune-inflamed tumors are characterized by high TCR clonality independent of GI [22]. Hence, future researchers should adopt a detailed set of criteria that consider the counting, subsets, and functional states of TILs when classifying tumor immunophenotypes. Personalized TR TCR-based adoptive T-cell therapy may offer a perspective for treatment-resistant immune-cold tumors.

Overall, a generalizable link between GI and tumor immunophenotypes remains elusive. A recent large-scale study spanning over thirty-one cancer types revealed that only a quarter of the participants displayed a positive correlation between mutational load and $CD8^+$ T cell infiltration, along with optimal ICB responses and prolonged overall survival [111]. In contrast, the remaining large proportion, including prostate and ovarian cancers that are often assumed to be TMB-H, and high-MSI glioblastoma, demonstrate an immune-excluded or -desert phenotype with unfavorable or even negative ICB response rates [38, 112].

Antigen presenting machinery dictating immune landscape (Fig. 3)

Antigen presenting cells (APCs), indispensable element of the immune system for capturing, processing, and presenting tumor antigens, are rate-limiting for T-cell priming and activation [113, 114]. Among the various APCs, DC are the most potent and are effectively engaged within the inflamed TIME [114]. In murine models, STING agonists have been shown to promote DC maturation and antigen presentation, converting the TIME into an immune-inflamed state [59]. However, a lack of DCs may be the primary reason for the immune-desert phenotype. According to a study on melanoma, the inflamed and non-inflamed subsets exhibited a comparable load of immunogenic antigens, while the latter cohorts were deficient in recruiting and activating Batf3-lineage DCs, the key cell type for the initial cross-priming of anti-tumor $CD8^+$ T cells, which implied that any malfunction in the APM could potentially impact the context of the TIME [94].

The APM are activated upon exposure to "danger signals", including pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) [115]. Typically, DC maturation signals are

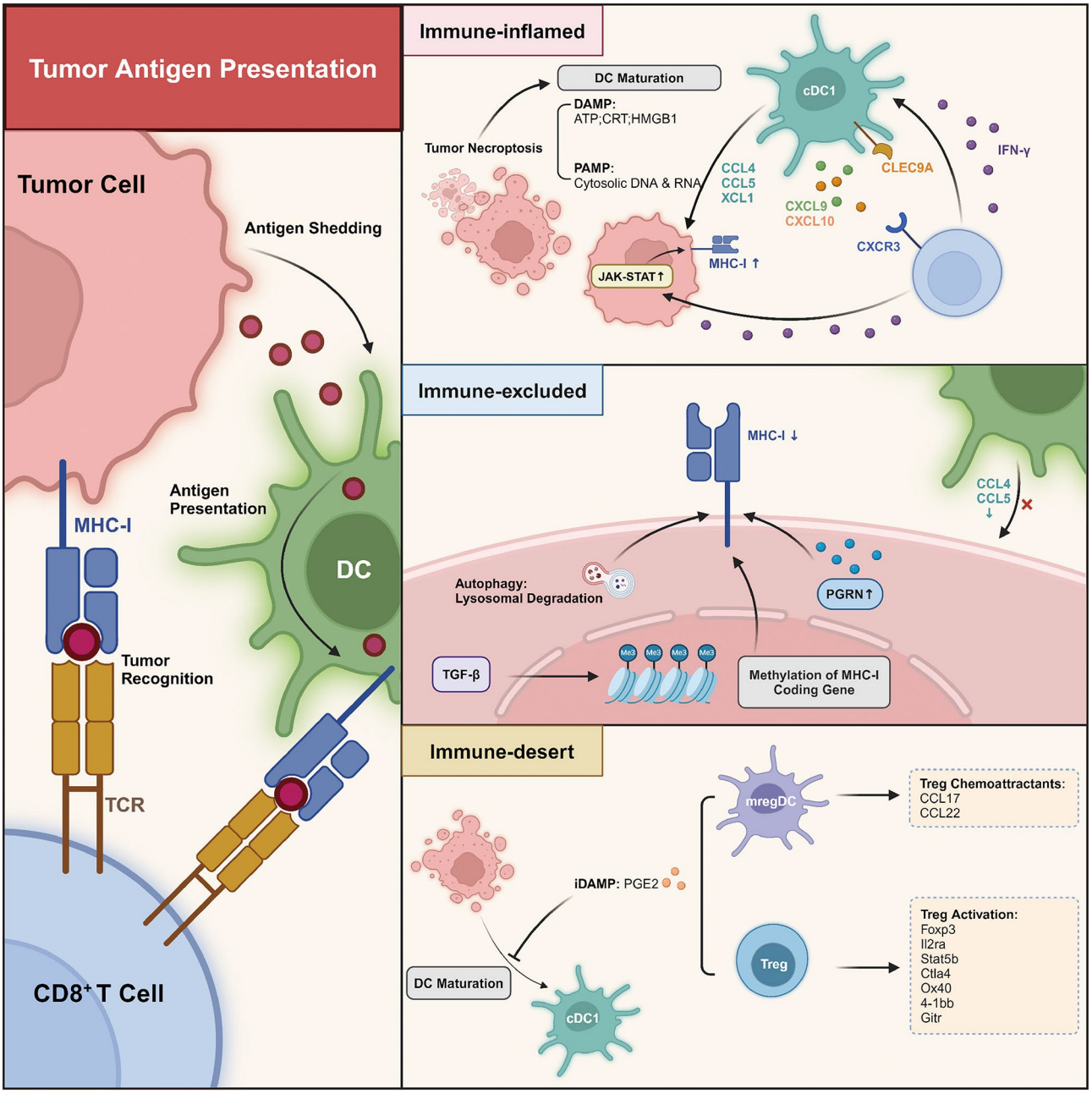

Fig. 3 Antigen Presentation Machinery Impact the Landscape of TIME. The antigen presentation machinery involves antigen shedding by tumor cells, this immunogenic signal is then captured and presented by DCs, which subsequently activate $CD8^+$ T cells and empower them to recognize and eradicate tumor cells. Elevated immunogenic cell death, specifically necroptosis, is a distinguishing feature of immune-inflamed tumors. Upon detecting DAMPs and PAMPs from dying tumor cells, DCs undergo maturation and are prepared to transmit information for $CD8^+$ T cells, in which the immune-enhancing $CLEC9A^+$ cDC1s play a major role. As an important mediator, cDC1s migrate to tumor nests through chemoattractant CCL4, CCL5, and XCL1, while simultaneously releasing CXCL9 and CXCL10 to recruit $CD8^+$ T cells. In turn, the increased IFN-γ signaling potentiates cDC1s while upregulates tumor MHC-I by triggering the JAK-STAT pathway, thereby forming a positive feedback loop for immune cell infiltration. The immune-excluded tumors generally employ "camouflage strategies" with reduced MHC-I expression. Epigenetically, elevated TGF-β promotes the methylation of MHC-I coding genes. MHC molecules are susceptible to lysosomal degradation via tumor autophagy and the presence of PGRN has been correlated with the down-regulation of MHC-I. Moreover, decreased DC chemoattractant also explains the decreased level of T cell infiltration. In the immune-desert TIME, iDAMPs, such as PGE2, can unbalance the antigen presentation machinery by promoting Treg activation, as well as by inducing mregDCs that facilitate Treg infiltration, which collectively lead to a barely inflamed TIME. *Abbreviations*: DC: Dendritic Cell; DAMPs: Pathogen-associated Molecular Patterns; iDAMPs: inhibitory Pathogen-associated Molecular Patterns; PAMPs: Damage-associated Molecular Patterns; CLEC9A: C-Type Lectin Domain Containing 9 A; PGRN: Progranulin; PGE2: Prostaglandin E2

released by dying tumor cells and act as cues for potential non-self-substances [116]. Subsequently, DCs move towards the draining lymph nodes (dLNs) where they process and load cancer antigens onto MHC-I molecules. This crucial step prepares DCs to present antigens to $CD8^+$ T cells [115].

Immune-inflamed tumors generally demonstrate upregulated gene expression of immunogenic cell death, particularly necroptosis. The release of DAMPs from necroptotic tumor cells can potentially amplify IFN-γ production and thus stimulates DC activity, which strongly correlates with the density of intra-tumoral $CD8^+$ T cells [117]. C-Type Lectin Domain Containing 9A (CLEC9A), a receptor present on DCs, is required to convey information from necroptotic cells to T cells [118]. In the inflamed TNBC subgroup, a high number of $CLEC9A^+$ DCs were found in close proximity to $CD8^+$ T cells, indicating the T-cell-activating role of DCs [58]. In contrary, inhibitory damage-associated molecular pattern (iDAMP), such as prostaglandin E2 (PGE2), has been found to dampen the immunogenicity of tumor necroptosis [119]. As observed in murine models of bladder cancer, iDAMP blockade (in the presence of celecoxib or a PGE2 neutralizing antibody) enabled DCs to skew towards immunogenic maturation and successfully transformed the immune-excluded TIME into a T-cell-inflamed pattern [120].

Tumors with immune-inflamed characteristics do not necessarily demonstrate a favorable therapeutic prognosis, as the biological paradox persists: active inflammation often parallels immunosuppression in the TIME [121]. This enigma has been elucidated by several hypotheses involving DCs. Maier et al. elucidated mregDC-Treg axis driven by PGE2-EP2/EP4 signaling. mregDCs, a newly deciphered DC population, are characterized by a homeostatic immunoregulatory gene signature that can restrain immune-enhancing cDC1 and induce anergy in effector T cells [122]. In TIME-inflamed Lewis lung carcinoma-bearing mice, the immune landscape can also be modulated by the regulatory node PGE2-EP2/EP4 signaling, which further potentiates mregDCs and elicits the amplification of CCL22 and CCL17 that expand and activate Tregs [123]. Indeed, maintaining a balanced ratio of cDC1: mregDC is critical for effective cancer elimination, even within an inflamed TIME. Secreted factors such as TGF-β, IL-6 and IL-10 have been shown to hijack this balanced regulatory mechanism, consequently inducing the TIME towards an immunosuppressive profile [124–126].

It has been reported that a subset of DCs migrates to the dLNs and aid in T cell cross-priming, while another group infiltrates the tumor nests to facilitate effector T cell homing and amplify the engraftment of TILs. Chemokines, such as CCL4, CCL5, and XCL1, released from a wide range of cell sources, are key chemoattractants for cDC1s to infiltrate from periphery lymphoid compartments into neoplasms [127, 128]. As observed in treatment-naïve advanced ovarian tumors, CCL5 levels were consistently associated with diffuse T-cell infiltration, which has been proposed to be a targetable factor in transforming immune-desert tumors [129]. Importantly, the dominant chemokines required by DCs to recruit $CD8^+$ T cells are those that engage with the chemokine receptor, CXCR3 [130]. In the immune-inflamed TIME, CXCR3-ligands (such as CXCL9 and CXCL10) are predominantly expressed by the $CD103^+$ DC population [131]. Aberrant activation of the tumor-intrinsic WNT pathway has been observed to exclude tumor-reactive T cells in melanoma and the immune-excluded subtype of TNBC, resulting in the near-complete absence of T cells within the tumor nest. Mechanistically, the blockade of T cell infiltration could be attributed to the inadequate recruitment of cDC1s and $CLEC9A^+$ DCs respectively, which was partly due to the impaired production of CCL4 and CCL5 [94]. Furthermore, β-catenin-expressing tumors also demonstrate failure to facilitate re-expansion of $CD8^+$ memory T cells. These findings provide insights into DC-chemoattractant-based reconstitution to address intra-tumoral DC deficiency and restore T cell migration into the tumor parenchyma. Accordingly, a preclinical lung cancer study leveraged nanoparticle-based delivery of CXCL9-11 plasmids towards the TIME, which promoted $CD8^+$ T cell infiltration and retarded tumor progression [132]. Meanwhile, studies by Zheng et al. and Terhorst et al. have shown that the upregulation of CCL5 or administration of an XCL1-based vaccibody (bivalent vaccine molecule) improved DC chemotaxis, thereby "heating" a scarcely immunogenic TIME into an "inflamed" TIME by attracting a high number of $CD8^+$ T cells [133, 134].

MHC-I molecules are "road marks" for cytotoxic T cells. The functional status of MHC may also contribute to tumor immunophenotypes. As observed in immune-inflamed tumors, elevated IFN-γ upregulates the components of MHC-I by triggering the JAK-STAT signaling pathway [135]. In the immune-excluded subgroup of ovarian cancer, the specific downregulation of MHC-I in the tumor compartment can be partially attributed to epigenetic regulation. Mechanistically, TGF-β promotes DNA methylation, while applying DNA methyltransferase inhibitor has been demonstrated to restore MHC-I in vitro [38]. Furthermore, HLA-I LOH is identified as an independent prognostic marker for patients with the "cold" subtype of TNBC [136]. With impaired DNA double-strand break repair, HLA LOH tumors produce higher levels of neoantigens; however, defective APM demonstrate limited immune selection pressure and an absence of cancer-killing cells within the TIME. As

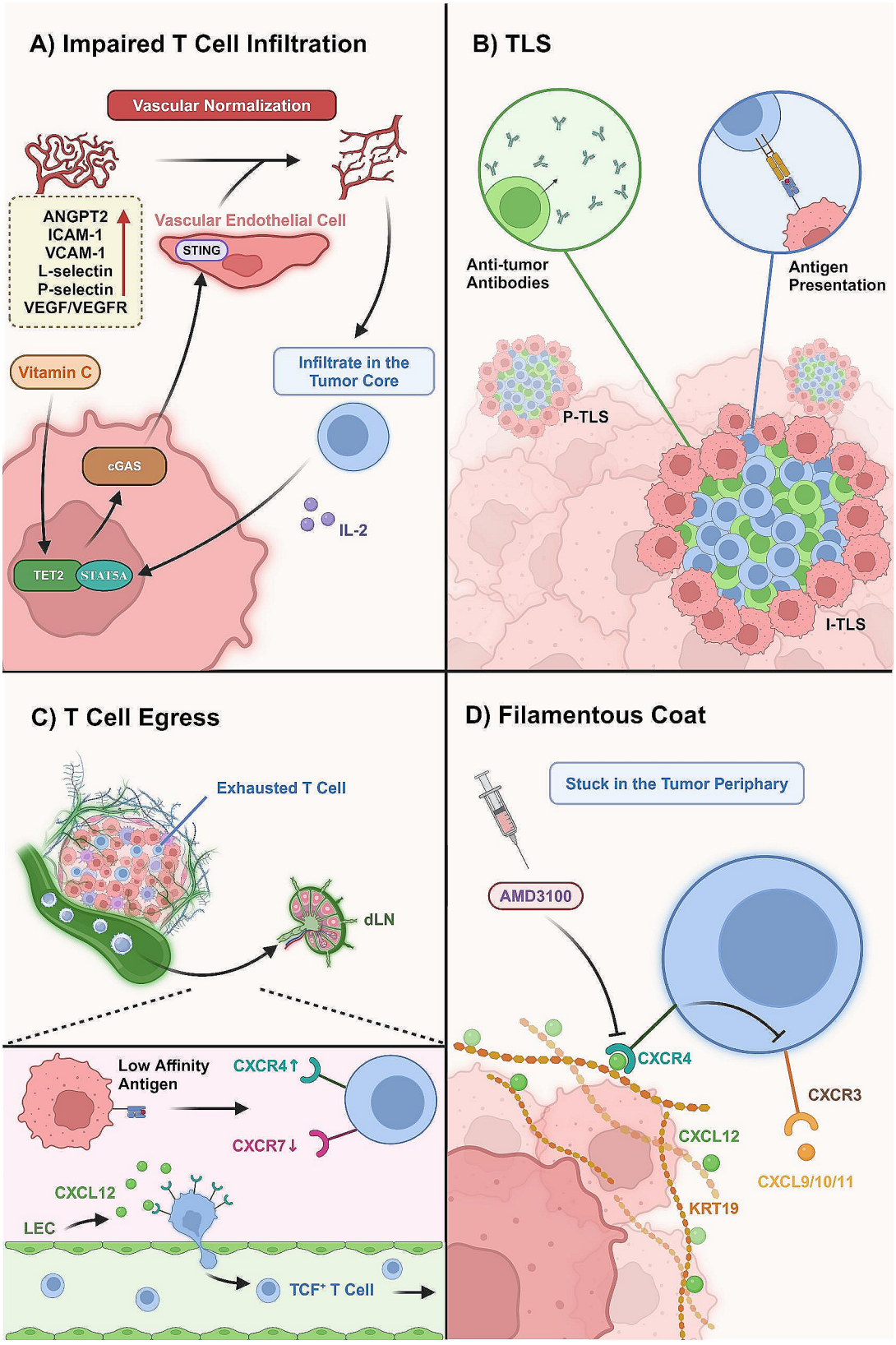

Fig. 4 (See legend on next page.)

(See figure on previous page.)

Fig. 4 Sustained Intra-tumoral $CD8^+$ T cell Infiltration is Integral for the Immune-inflamed TIME. (A) The distorted and leaky tumor vasculatures impede efficient $CD8^+$ T cell infiltration. While administration of Vitamin C breaks the vicious circle to normalize vasculatures via the cGAS-STING crosstalk between tumor and vascular endothelial cells. The consequent infiltration of $CD8^+$ T cells secrete IL-2 to enhance the crosstalk mechanism, thereby creating a positive feedback loop. (B) TLS, the ectopic lymphoid aggregate, provide alternative infiltration routes for T cells. The specific location and composition of TLSs are determinant for tumor immune phenotypes. I-TLSs are proximate to tumor nests, where anti-tumor antibodies from $CD4^+$ T cells and cytotoxicity of $CD8^+$ T cells are effectively engaged to eliminate tumor cells, unless TLSs were immature with abundant immunosuppressive Tregs. (C) The T cell egressing mechanism is mediated by lymphatic vasculatures. The upregulated CXCL12-CXCR4 axis between LECs and $CD8^+$ T cells expels the $TCF1^+$ stem-like population towards dLNs while the exhausted T cell subsets are retained intra-tumorally. This procedure is modulated by tumor-antigen affinity. Specifically, high-affinity antigens downregulate CXCR4 and instead upregulate CXCR7 in $CD8^+$ T cells, thereby facilitating T-cell retention. (D) A filamentous network of CXCL12-KRT19 heterodimers coat tumor cells to exclude T cell infiltration. The upregulation of CXCL12-CXCR4 interplay disrupts CXCR3, which is integral for chemoattractant to recruit $CD8^+$ T cells. The CXCR4 inhibitor, AMD3100, has been proved to expand intra-tumoral T cell infiltration. *Abbreviations*: cGAS: cyclic GMP-AMP Synthase; STING: Stimulator of Interferon Genes; TLS: Tertiary Lymphoid Structure; I-TLS: Intra-tumoral Tertiary Lymphoid Structure; LEC: Lymphatic Endothelial Cell; TCF1: T Cell Factor 1; dLNs: draining Lymph Nodes

human malignancies progress, tumors may proactively adopt multiple mechanisms to restrict APM and "camouflage" themselves virtually invisible to cytotoxic cells. MHC molecules are susceptible to lysosomal degradation via tumor autophagy. Treatments targeting autophagy can reverse this process with increased CTL infiltration, as shown in a PDAC mouse model [137]. Progranulin (PGRN), a crucial mediator in neurodegenerative diseases, has recently emerged as a TIME modulator. A study of patients with PDAC identified a correlation between PGRN positivity, reduced expression of MHC-I, and deficient infiltration of $CD8^+$ T cells. This was further corroborated by PGRN blockade, as it recovered the APM and boosted the cytotoxicity of $CD8^+$ T cells [138]. While complete eradication of MHC-I may seem to be a favorable strategy for tumors to maintain a non-inflamed TIME, the immune system has a crucial checkpoint for the loss of MHC-I presentation. Specifically, NK cells act as the "monitor" via "missing self" recognition. In melanoma, the number of NK cells was found to be indicative of both anti-PD-1 efficacy and prognosis, which underscores their potential role against MHC-I-deficient non-inflamed tumors that are refractory to T cell-based regimens [127]. Apart from yielding antigen-loss or MHC-I-negative variants, immune pressure can also select for the outgrowth of tumors cells with activated immune-evasive oncogene pathway such as WNT/β-catenin. Specifically, immune-mediated selection for antigen-loss subclones may occur exclusively in inflamed tumors, which indicates the potential transformation into immune-excluded or immune-desert TIME over time [131].

Overall, any defect in the APM system may lead to a barely inflamed TIME in tumors with high TMB. Indeed, evidence that antigen load may be indistinctive across inflamed-, excluded-, and desert-TIME has holds grounds for exploring novel and general therapeutic strategies to restore DC function and T cell infiltration [94]. Mature DCs are mandatory for an effective anti-tumor response, and focusing on this step in the immune cycle could offer viable treatment options for patients with non-inflamed tumors. DC vaccination therapy yielded encouraging results in a representative immune-desert malignant pleural mesothelioma, as demonstrated in the DENIM trial (NCT03610360) [139]. DCs were ex-vivo cultured and activated before being administered to inflame the TIME, which unleashed the horizon for combination immunotherapy using DC therapy as a backbone.

T-cell trafficking and infiltration within the TIME (Fig. 4)

The immunological state of a tumor depends on the extent to which effective tumoricidal cells can access and persist in the tumor parenchyma. The tumor-directed accumulation of immune cells entails sequential interactions that involve tethering, adherence, and migration across specialized post-capillary venules, namely, high endothelial venules (HEV) [140, 141]. To achieve the efficient and durable elimination of malignant cells, cytotoxic T cells must be retained in the tumor parenchyma. The TIME is generally characterized by aberrant destabilized vascularization. Concerted crosstalk between CTLs and tumor vasculature is a determinant for T cell trafficking and infiltration [60].

Emerging evidence highlights the significance of normalized blood vessels in creating an immune-active TIME. Immune-excluded tumors typically contain fewer TILs by sequestering T cells away from their targets. ANGPT2, also known as vascular destabilizing factor angiopoietin-2, was recently observed to destabilize the peripheral vasculature in immune-excluded melanoma, thereby restricting intra-tumoral T-cell accrual [142]. Furthermore, immune-excluded tumors express higher levels of endothelial adhesion molecules (AMs) such as vascular cell adhesion molecule 1 (VCAM-1) at the tumor periphery. The difference in spatial AM expression is postulated to drive T cells to be maintained at the periphery rather than at the tumor core. Interestingly, anti-ANGPT2 treatment improved vascular integrity and decreased AM discrepancy, especially VCAM-1 and L-selectin, which released sequestered T cells from the periphery into the central tumor areas and reversed the immune-excluded phenotype [142]. Targeting the abnormal tumor vasculature system offers promising perspectives for the development of an inflammatory

phenotype. A recent murine model-based investigation of liver cancer revealed that Vitamin C is a potential therapeutic agent. Intraperitoneal administration of Vitamin C stimulates teneleven translocation-2 (TET2) and upregulates tumor cyclic GMP-AMP synthase (cGAS). As a result of crosstalk, the STING signaling pathway in endothelial cells is activated, leading to the normalization of tumor vasculature and an increase in transendothelial CTL migration. Consequently, IL-2 produced by infiltrating lymphocytes stimulates tumor STAT5A signaling, which, in turn, synergizes with TET2 to epigenetically elevate tumor cGAS expression, thereby establishing a positive feedback loop [143]. Furthermore, VEGF/VEGFR2 signaling is the most potent driver of dysregulated angiogenesis and has been identified as the key to restore the inflamed TIME [144, 145]. As evidenced by studies of breast and pancreatic cancers, inhibition of VEGF/VEGFR2 signaling improved T cell availability at the tumor core with "vascular normalization" and sprouted HEV [146–149]. In addition, neoropilin-1, a VEGF co-receptor found in tumoral Tregs, has shown double-effect therapeutic benefits. The Treg-specific blockade of neoropilin-1 may attenuate immunosuppressive Tregs and normalize dysregulated angiogenesis, leading to improved $CD8^+$ T cell infiltration [150]. Moreover, preclinical investigations have proven that the dual neutralization of ANGPT2 and VEGF altered tumor vasculature effectively and contributes to enhanced antitumor immunity [142].

Additionally, the lymphatic system plays an integral role in shaping the TIME, as supported by the positive correlation between lymphatic vessel density and infiltrated cytotoxic T cells in patients with CRC [151]. Steele et al. illustrated that tumor-associated lymphatic vessels can instruct intra-tumoral $CD8^+$ T cell repertoire in melanoma [152]. During acute inflammation, CCL21-CCR7 ligation allows for the drainage of naïve T cells back into circulation to maintain immune cell homeostasis [153, 154]. Nevertheless, in tumors, T cells employ a CCR7-independent mechanism to exit through the inflamed lymphatic vasculature, thereby transforming the TIME into an immune-excluded state. Tumor-associated lymphatic endothelial cells upregulate CXCL12 expression, which then interacts with CXCR4 in T cells. Activation of the CXCL12-CXCR4 axis expels $TCF1^+$ stem-like T cells, which are integral to sustain the intra-tumoral effector T-cell response, and sequesters them at the tumor periphery, where they are likely to egress [152]. This novel T-cell egressing mechanism is tuned by tumor-antigen affinity. Specifically, high-affinity antigens can downregulate CXCR4 by effector $CD8^+$ T cells and instead upregulate the CXCL12 decoy receptor CXCR7 to promote T cell retention. However, this selectively enriched T-cell group was mostly $PD\text{-}1^+$ $TIM3^+$ and $LAG3^+$, indicating compromised functionality of retentive T-cells. Similarly, targeting CXCL12-producing fibroblasts unleashed $CD8^+$ T cell immunity and persistent tumor control [155]. The interaction between CXCL12 and CXCR4 could otherwise act physically to exclude T cells. As shown in PDAC, CRC, and BC, a filamentous network of CXCL12-KRT19 heterodimers is assembled to coat tumor cells. The CXCL12–KRT19 coating cross-links CXCR4 receptors on adjacent T cells and stimulates a CXCR4 "stop" signal, which plays a dominant role in suppressing T-cell motility and leading to T cell exclusion [156]. As a corollary, targeting the CXCL12-CXCR4 axis could either reduce T cell egression or destroy the filamentous coat that impedes infiltration, thereby expanding the available T cells for immune-noninflamed tumors. Administering AMD3100, a clinically approved CXCR4 inhibitor, has proved increased T cell accumulation within the tumor core [155].

Consistent with these findings, in vivo photoconversion-based analyses of murine models longitudinally examined immune trafficking and egression, which revealed that the majority of $CD8^+$ T cells in the tumor deposits undergo an exhausted phenotype within a 72-hour time frame. The intra-tumoral pool of $TCF\text{-}1^+$ $PD\text{-}1^+$ cells is continuously replenished by newly entering cells, whereas this population is not retained but amongst the T cells that recirculate to the dLNs, where they can evade chronic antigen exposure to maintain stem-like characteristics [157]. Furthermore, in immune-inflamed TIME, lymphatic vessels can initiate a negative-feedback program upon detecting T cell-derived IFN-γ. Subsequently, elevated lymphatic PD-L1 constraints further CTL accumulation by trapping them in peripheral tissues and creating an excluded infiltrate phenotype [158]. Therefore, it is conceivable that the blockade of PD-L1 could on the one hand rejuvenate the "quantitative-cold" TIME, while simultaneously ensuring sustained infiltration of effector CTLs into the tumor parenchyma.

Tertiary lymphoid structures (TLSs) provide an alternative and efficient route for T cells to migrate towards neighboring tumor nests and proliferate within the TIME, circumventing the conventional vasculature trafficking programs [159]. TLSs are organized ectopic lymphoid aggregates that develop during carcinogenesis and comprise T-cell and follicular B-cell zones [160, 161]. This specialized structure is associated with promising results towards prognosis and ICB therapies, as it provides tumor-responsive T cells with locations for priming, proliferation, and direct migration towards the tumor parenchyma [159, 162]. Indeed, high TLS densities are significantly associated with favorable clinical outcomes across a wide array of solid tumors, including gastrointestinal tumors [163], NSCLC [164], hepatocellular carcinoma (HCC) [165], melanoma [166], BC [167], and CRC

[168]. An analysis of NSCLC revealed that patients with stage III disease exhibit fewer TLSs than those with stage II disease, indicating that tumor cells may evade immune responses by disrupting TLS formation during progression [169]. Furthermore, a large-scale retrospective analysis has shown that the number of intra-tumoral TLSs is positively correlated with ICB therapy efficacy, independent of PD-L1 expression [170]. Specifically, for TNBC, a higher abundance of TLS was observed at the periphery of both the inflamed and excluded subtypes, except for the desert immunophenotype [58]. Leiomyosarcoma and intrahepatic cholangiocarcinoma (iCCA), which were previously considered immune-cold and devoid of effector immune cells, exhibited distinctively "hot" subsets characterized by higher frequencies of TLSs [171, 172]. A closer examination suggested that an elevated percentage of stromal cells may impede TLS formation in immune-excluded tumors, consequently reducing the anti-tumor immunity [173]. The function of TLSs may vary across cancer types and depend on spatial arrangements, which classify TLSs into intra-tumoral TLS (I-TLS) and peritumoral TLS (P-TLS). In desmoplastic melanoma, I-TLSs are in closer proximity to tumor cell clusters, contributing to an immunologically inflamed TIME, unlike in non-desmoplastic melanoma, where TLSs are embedded peritumorally [174]. In contrast, in CRC, a high density of P-TLS confers an immune-active TIME and improved prognostic value, whereas I-TLSs are perfused by immunosuppressive Tregs [173]. Ding et al. revealed that the maturity of TLS is reliant on $CD4^+$ $Bcl6^+$ follicular helper T cells [172]. It is speculated that in the absence of this cell group, P-TLS may remain immature and dysfunctional, potentially destroying the formation of an inflamed TIME by expanding Tregs in I-TLSs. In addition to T cells, the role of TLS-resident B cells in orchestrating the immune-inflamed TIME has recently emerged as a focal point of research [175–177]. Interestingly, Vanhersecke et al. proposed that the presence of mature TLS correlated with favorable outcomes in tumors featuring high $CD8^+$ T cell infiltration, whereas patients with limited $CD8^+$ T cell infiltration failed poorly, irrespective of TLS status [170]. This observation indicates that $CD8^+$ T cells are essential but not adequate for initiating a persistent anti-tumor immune response, necessitating close cooperations with B cells. Researchers have uncovered high expression of both MHC class I and class II molecules in B cells within TLSs, highlighting their proficiency in antigen presentation [166]. Moreover, TLS can facilitate the differentiation and maturation of B cells into plasma cells, which can produce tumor-specific antibodies that attach to tumor cells [178]. Investigations into PDAC have elucidated that this process can be explained by the synergistic interactions involving TGF-β signaling, the expression of the B cell chemoattractant CXCL13 by tumor-reactive T cells, and the supportive role of fibroblast-derived TGF-β, which collectively enhance the activation of B cells [179]. Further analysis of ccRCC suggested that these mature plasma cells within the TLS are guided by $CXCL12^+$ fibroblasts to migrate deeper into tumor foci while producing IgG and IgA antibodies and boosting anti-tumor effects [180]. The subsequent formation of antigen-antibody complexes could be internalized by DCs and favor efficient antigen presentation to T cells, which allows enhanced activation of $CD8^+$ T cells, particularly in the context of ICB therapies [181–183]. Collectively, these findings add an important facet to leverage the TLS as a biomarker for identifying potential patients who may benefit from ICB therapies. Currently, clinical trials in patients with sarcoma are exploring this innovative approach (NCT02406781; NCT04095208) [170]. According to a study by Hammerl et al., however, the presence or density of TLSs in TNBC are independent of survival rates, even when stratified by immunophenotypes, which warrants pertinent research to determine their contribution in shaping the TIME [58].

To date, it is unclear whether the egress mechanism of lymphatic vasculature is a bone or bane because it not only transports functional T cells away from tumors but is also likely to reinvigorate or recirculate the suppressed, exhausted, or naïve T cells, though to a relatively lesser extent. Furthermore, infiltrating T cells migrate to distant metastatic tumors and dLNs, underscoring a mechanism by which tumor-experienced effector T cells boost tumoricidal efficacy at secondary tumor sites [184]. Techniques such as photoactivation strategies and advanced imaging are emerging and are supportive of unraveling the advantageous or detrimental effects of T cell egress from intra-tumoral regions [185].

Immune-suppressive cells extinguishing immune inflammation

The cancer ecosystem recruits and domesticates an array of immunoregulatory cells to mold tumor immune phenotypes by interacting with various components within the TIME. For example, melanoma and $PD\text{-}L1^+$ myeloid cells, particularly macrophages and DCs, collaboratively form thin and continuous sheaths along the invasive border, thereby excluding CTLs [186]. In general, the major immunoregulatory cell populations, including myeloid-derived suppressor cells (MDSCs), neutrophils, tumor-associated macrophages (TAMs) and Tregs, play a significant role in reshaping the TIME either by quantitative superiority over cancer-killing cells or by modulating CTLs through diverse mechanisms [187].

MDSCs are a heterogeneous population of immature myeloid cells at various differentiation stages and can be categorized into monocytic MDSCs (M-MDSCs) and polymorphonuclear MDSCs (PMN-MDSCs), according

to their phenotypic and morphological similarities to monocytes and granulocytes, respectively [188, 189]. As the key mediator orchestrating immunosuppression across solid tumors, the intra-tumoral enrichment of MDSCs has been linked to an unfavorable prognosis [190]. Indeed, the progenitors of MDSCs activate multiple signaling pathways that promote their amplification and inhibit further differentiation, with the majority converging on the JAK-STAT pathway [191], which upregulates immunosuppressive mediators such as reactive oxygen species (ROS), arginase, and inducible nitric oxide synthase (iNOS) [192]. Tumor-mediated PMN-MDSCs primarily inhibit effector T cells via ROS, whereas M-MDSCs mainly suppress T cells via arginase and iNOS [189, 193]. MDSCs are negatively associated with CTLs, where decreased MDSCs result in an increased intratumoral frequency and amplified tumoricidal effect on $CD8^+$ T cells [194, 195]. An investigation of PDAC has unveiled that CXCL1 is a molecular "switch" between the inflamed and the non-inflamed TIME. As a tumor cell-intrinsic regulator, non-inflamed tumor-derived CXCL1 signaled to increase the infiltration of MDSCs and simultaneously expels DCs and T cells, driving resistance to immunotherapy [196]. CCL2 triggers the release of tumor-toxic granules in $CD8^+$ T cells and NK cells, whereas MDSC-produced reactive nitrogen species can nitrify CCL2 (N-CCL2) [197]. Studies on human colon and prostate cancers demonstrated that N-CCL2 entraps T cells in the stroma, creating an immune-excluded TIME [198]. However, the disruption of CCL2 nitration promotes favorable lymphocyte infiltration in preclinical models. Additionally, plasma membrane expression of the metalloprotease ADAM17 by M-MDSCs has been proposed to downregulate L-selectin in T cells, thereby impairing their extravasation from the lymphatic system and migration into tumor lesions [199]. This scenario is further supported by a study using a B16 mouse model, which showed that L-selectin deficiency resulted in a limited number of tumor-infiltrating T cells [200]. Moreover, compared to circulating MDSCs, tumor-residing MDSCs demonstrate higher PD-L1 expression in various murine models and cancer patients, which interacts with T cells to induce their anergy [201–203]. Therefore, strategies to counteract the tumor-supportive activities of MDSCs may offer significant therapeutic benefits. In a preclinical model of immune-desert bladder cancer, the concomitant application of MDSC blockade with radiotherapy synergistically enhanced tumor-infiltrating CTLs, underscoring a breakthrough in MDSC-targeted therapy for remodeling the tumor immunophenotype [204].

Tumor cells may exploit neutrophil biology in their own interest, including hijacking the formation of neutrophil extracellular traps (NETs). NETs are web-like chromatin structures composed primarily of DNA extruded from the nuclei of neutrophils [205]. Research has indicated that the NET-DNA structure serves as a protective shield against cytotoxicity for tumor cells by physically blocking direct interactions with tumoricidal cells [206]. Confocal imaging of tumors has revealed that NETs form a barrier at the tumor-stroma interface. In recent studies, researchers have discovered that a direct chemotactic stimulant, the tumor-produced cytokine Chitinase-3-like 1 (Chi3l1, YKL-40/CHI3L1 in humans), promotes NET formation in TNBC [207]. Chi3l1 co-opts neutrophils to mediate stromal restriction of T cells, whereas ablation of Chi3l1 ameliorates tumor growth and enhances the ICB response. In the context of pancreatic cancer, γδ T cell-secreted IL-17 contributed to NET formation, which further suppressed $CD8^+$ T cell recruitment and conferred the immune-excluded phenotype of PDAC [208], while IL-17 neutralization has allowed spatial redistribution of CTLs that favored proximal migration to tumor nests. Furthermore, the presence of PD-L1 in NETs has a broader impact by shifting T cells into the exhausted phenotype, inducing a "quantitative cold" state [209]. As shown in invasive bladder cancer, the administration of DNase I to digest NETs also inflamed the TIME with effective $CD8^+$ T cells accumulation [210].

TAMs are also important players in the TIME, representing the predominant population of tumor-infiltrating immune cells (over 50%) in various cancer types, such as melanoma, renal cancer, and CRC [211]. TAMs are highly plastic cells that exhibit functional heterogeneity in response to various stimuli [212]. Typically, TAMs can be categorized into two classes: M1 and M2 [213]. Classically activated M1 macrophages are primarily involved in proinflammatory responses. Similar to DCs, M1 TAMs are capable of phagocytosing tumor-associated antigens, albeit to an inferior extent [214], and can serve as APC to induce specific anti-tumor immune responses [213]. Conversely, M2 TAMs undergo alternate activation and are primarily involved in anti-inflammatory responses. Both M1 and M2 TAMs are present throughout all stages of tumor development, with M1 TAMs dominating in the early stages and M2 TAMs prevailing as the tumor advances [215]. M1 TAMs progressively shift towards M2-polarized TAMs with tumor progression, whereas an elevated frequency of M2 TAMs correlates with an unfavorable prognosis. Intravital imaging studies of the TIME indicated that antigen-specific $CD8^+$ T cells tended to localize in TAM-rich areas and positive correlations existed between the infiltration and exhaustion of $CD8^+$ T cells with TAMs [216, 217]. Research of melanoma models indicated that the highest level of exhaustion in cytotoxic T cells is present near the macrophage barrier, where macrophages are plentiful and have increased opportunity to interact with T cells within the "effective interaction distance", typically defined within a

radius of less than 20 μm [218]. TAMs and $CD8^+$ T cells are shown to engage in a weakly stimulatory, yet persistent antigen-specific synaptic contacts that initiate T cell exhaustion program and may lead to a "quantitative cold" state [214]. Thereafter, exhausted T cells concurrently form a self-enforcing feedback loop, which is exacerbated under hypoxic conditions, particularly at the tumor core, to expand the intra-tumoral pool of TAMs via secreting CSF1, CCL3-5. Moreover, TAMs, by CCL18, can function to recruit naïve $CD4^+$ T cells with the potential to differentiate into Tregs via secreting TGF-β and IL-10 [219, 220]. As reported in studies on ovarian cancer patients and murine gastric cancers, TAMs can attract mature Tregs into the TIME through the production of chemokines CCL20 and CCL22, which cooperate to suppress $CD8^+$ TILs [221, 222]. Furthermore, a recent investigation on CRC has revealed that the TIME specifically endowed TAMs with elevated inhibitor of differentiation 1 expression, which interacted with STAT1 to suppress the chemoattractant CCL4, thereby excluding $CD8^+$ T cells from tumor parenchyma [223]. Macrophage-coated tumor clusters (MCTC), distinctive spatial structures characterized by abundant macrophages surrounding tumor clusters, have been identified in HCC. MCTC is a prevalent structure that was also observed in 35.5% of BC samples and 23.5% of lung squamous carcinoma samples, which impedes $CD8^+$ T cell infiltration and relegates them to the tumor periphery [224]. In terms of the mechanism, the tumor-derived macrophage-associated lectin Mac-2 binding protein could orchestrate the recruitment of the M2 macrophage subpopulation and enhance cellular adhesion, leading to the formation of this robust immunosuppressive barricade. Therefore, converting M2 TAMs into anti-tumor M1 subtypes is a promising immunotherapeutic approach for the treatment of solid tumors [225]. The application of chimeric antigen receptor macrophages (CAR-Ms) in preclinical studies has demonstrated M1 TAM polarization, improved phagocytosis of tumor cells, and restored tumoricidal function of $CD8^+$ T cells [226, 227]. Compared with CAR-T therapies, CAR-M has particular advantages in terms of its capacity to migrate and penetrate the immunosuppressive TIME of solid tumors [228]. However, this innovative technology remains immature in the clinical setting. In contrast to the preclinical results where CAR-Ms reprogrammed the TIME, the TIME was capable of steering tumor-resident CAR-Ms towards a tumor-supportive phenotype, highlighting the need for further exploration into the underlying mechanism [229].

Immune-suppressive cells are intricately linked. Research of melanoma-bearing mice has shown that indoleamine 2,3-dioxygenase (IDO) can orchestrate both local and systemic immunosuppression through the expansion, infiltration, and function of MDSCs within the TIME, in a manner dependent on Treg recruitment [230, 231]. Interestingly, inhibiting IDO or depleting Tregs decreased intra-tumoral MDSCs and reversed immune suppression. Studies have revealed a correlation between MDCSs and Tregs in multiple cancers such as metastatic prostate cancer, glioblastoma, and renal cell carcinoma [232]. Moreover, IDO-deficient mice demonstrate retardation of lung tumor progression, with MDSCs exhibiting impaired immunosuppressive ability due to IL-6 attenuation [233]. Previous research has also proposed that IDO can induce and activate Tregs; however, the mechanisms underlying Treg-mediated MDSC recruitment and activation are not completely understood [234]. In addition, Treg-DC interactions have been shown to disrupt CTL-DC engagement, leading to unfavorable enrichment of inactivated T cells. Treg's contact-dependent ligation with DCs also generates metabolic destructions that contribute to a "quantitative cold" TIME, the consequent IDO production by DCs catabolizes the essential amino acid into suppressive metabolites including kynurenine, which in turn activates Tregs and MDSCs [235]. Administering the IDO1 inhibitor, LW106, to melanoma cells resulted in a reduction in tumor-associated stromal cells and collagen deposition, consequently eliciting CTL infiltration [236]. These findings provide a compelling rationale for using IDO inhibitors as adjuvants to convert immune-cold tumors by highlighting the significant association between immunosuppressive cells and IDO in the TIME.

Barriers blocking T-cell infiltration

Currently, the exploration into how physical traits of cancer impact the immune landscape is still in its infancy. Solid stress, a compressive mechanical force mediated by the proliferation of cancer cells and desmoplasia of the ECM, has been shown to deter lymphocyte ingress and facilitate immune exclusion [237]. As measured in metastatic lymph node lesions, solid stress surges towards the lesion center. This modification of the TIME impairs lymphocytic trafficking by reducing the number of HEVs, particularly those expressing peripheral node addressin, the lymphocyte-homing receptor on endothelial cells. Immune exclusion is primarily observed in tumors with a collagen-rich ECM that features compact and linearly aligned fibers in the tumor stroma [238]. As shown in PDAC, stromal components account for >90% of the tumor mass [239]. Analysis of human using real-time imaging of T-cell dynamics has revealed dense fibers oriented parallel to the interface between the tumor and stroma [34]. This distorted stromal architecture creates a rigid barrier that peritumorally compartmentalizes the cancer-attacking cells. It also obstructs the diffusion of cytokines and chemokines, which are crucial to recruit and activate T cells. Moreover, T cells may adhere to

dense collagen, which could spark rapid motility along the collagen highway, rendering them distracted from the durable and serial killing of their targets [240]. Additionally, collagen can function as an immunosuppressive ligand for leukocyte-associated immunoglobulin-like receptor-1, curtailing cytotoxicity and inducing T-cell exhaustion [241]. Eliminating discoidin domain receptor 1, the collagen receptor responsible for collagen fiber realignment, promotes CTL infiltration in murine models of immune-excluded tumors such as metastatic urothelial cancer and TNBC [33, 242]. In melanoma, the administration of recombinant hyaluronan and proteoglycan link protein 1 led to a highly "basket-weaved" ECM structural pattern, which closely resembled that of normal epithelial tissues and was associated with increased T cell infiltration [241, 243]. The abundance of metalloproteinases, which are critical enzymes involved to degrading collagen and restructuring ECM fibers, was found to have an elevated infiltration advantage [244]. Alternatively, metalloproteinase-cleaved collagen fragments also activate integrin-dependent T cell motility, indicating that collagen elements can create chemotactic gradients that guide CTLs towards intra-tumoral areas [245]. However, it is yet to be determined whether collagen-degraded fragments can mobilize T cells at a high velocity and disrupt their engagement with cancer cells, or whether they can guide them to tumor cell-dense areas. Overall, these findings merit further attention regarding the critical role of ECM modulation processes in immune cell distribution, and whether targeting them could guarantee more frequent interactions with tumor cells remains unknown.

Cancer-associated fibroblasts (CAFs) play a prominent role in ECM deposition and remodeling that is endemic to immune-excluded tumors [246]. Transformed from quiescent normal fibroblasts, this immune population exhibits distinguished propagation and migration abilities. In fact, CAFs strongly demonstrate inter- and intratumor heterogeneity. Fibroblasts expressing fibroblast activation protein (FAP) are responsible for producing and organizing fibrous materials (such as fibronectin and collagen), thereby driving T cell marginalization and restricting their contact with cancer cells [247]. It has been noted that ECM-associated functions are predominantly executed by FAP^+ CAFs, which are identified in regions where the matrix is densely deposited [34]. A recent study of NSCLC discovered multiple layers of FAP^+ CAFs surrounding the tumor border, driving T-cell marginalization through the deposition and alignment of type XI and XII collagens. Another distinctive CAF subset with positive expression of myosin heavy chain 11 lines around a single layer and was strongly associated with immune exclusion [248]. These CAF subpopulations are characterized by high levels of periostin (POSTN) as well. Pan-cancer analysis of TCGA database has provided evidence that individuals with high POSTN or FAP expression are associated with a high Tumor Immune Dysfunction and Exclusion (TIDE) score, indicating greater potential for immune evasion and unfavorable prognosis from immunotherapy [249]. In murine BC models, nitric oxide underlies the stromal effects of CAFs featuring FAP and podoplanin (PDPN) positivity. Mechanistically, nitric oxide generated by FAP^+ $PDPN^+$ CAFs initiates TCR nitration in neighboring T-cells, which consequently leads to desensitization [250]. Similarly, N-CCL2 is implicated in the peritumoral entrapment of CTLs in the stroma. However, CAF heterogeneity may explain the contradictory outcomes of previous studies on stromal depletion. In PDAC, the composition of the immune cell infiltrates is dictated by two distinct CAF subtypes: $POSTN^+$ CAFs and $PDPN^+$ CAFs [251]. Tumors lacking $PDPN^+$ CAFs feature an immune-cold phenotype, and tumors with an adequate presence of $PDPN^+$ CAFs are associated with T cell infiltrates, unless abundant with $POSTN^+$ CAFs, which preferentially favor macrophage chemotaxis by activating the Akt signaling but exclude T cells from infiltration [249]. Consistently, the positive correlation of $PDPN^+$ CAFs and intratumoral T cells has been observed in TNBC, in which the immune-inflamed subtype is predominant [252]. Nevertheless, genetic ablation of CAF-derived POSTN has demonstrated accelerated tumor growth because it is essential for the formation of tumor capsules, indicating that certain $POSTN^+$ CAFs may be protective against tumor progression [253].

Senescence is a hallmark of cancer [254]. In addition to the heterogeneity of CAFs, the contribution of senescence programs should be carefully determined. Evidence suggests that senescent CAFs, which can be derived from various CAF subpopulations, reshape the TIME via an intricate tumor-CAF interplay. Essentially, the evolution of the senescent TIME is inextricably correlated with a shift in fibroblast behavior [255]. Senescent cancer cells have limited or null proliferative capacity; however, they emit a collection of pleiotropic cytokines, chemokines, growth factors, and matrix-modifying factors, which are referred to as the senescence associated secretory phenotype (SASP), to directly initiate stromal cell senescence [256, 257]. In the context of tumor progression, CAFs experience long-term induction of the SASP, which leads to immunosuppressive cell infiltration, including MDSCs, Tregs, and M2 macrophages [258, 259]. In addition, a study on ccRCC showed that immune exclusion-related signaling activity is upregulated along with the activation of the senescent program in CAFs, resulting in adverse prognostic implications [260].

Of note, TGF-β is a pivotal upstream mediator in CAF-associated exclusionary stromal reaction. Immune-excluded tumors including CRCs and urothelial cancers

have demonstrated increased levels of TGF-β-driven CAF gene expression program [33, 261]. In a model of pancreatic cancer with selective Treg deletion, the absence of Treg-derived TGF-β1 hinted towards decreased collagen synthesis by CAFs, subsequently leading to a greater T cell influx [262]. Beyond Tregs, TGF-β can be produced by various cell subsets within the TIME, including tumor itself and CAFs [261]. In addition, cancer-derived exosomes also carry nucleic acids or proteins such as surface-bound TGF-β1 to promote CAF generation [261]. Congruently, in the typical hypoxic TIME, increased TGF-β stimulates CXCL12/CXCR4 signaling via HIF-1α in both cancer cells and CAFs [263], and the elevation of CXCL12/CXCR4 axis serves the downstream role to recruit and activate CAFs, thereby driving matrix production and subsequent stromal T-lymphocyte exclusion [264]. The administration of AMD3100, a clinically approved CXCR4 inhibitor, has been shown to decrease fibrosis and alleviate solid stress that physically repels T cells in the stroma [265]. Furthermore, the ROS-producing enzyme NADPH-oxidase-4 (NOX4) is also recognized to act downstream of TGF-β1 and modulates CAF differentiation in multiple cancers. Investigations applying GKT137831 (Setanaxib), a small molecule inhibitor of NOX4/1, suggested that targeting NOX4 can revert CAFs to the "normalized" phenotype and increase intratumoral $CD8^+$ T cell density as a result [266]. In tumor models featured with TGF-β activity in CAFs, using pan-TGF-β antibody has successfully enabled T cell infiltration [33]. A study interrogating TGF-β neutralization has revealed significant reduction of ECM density accompanied with a shift of tumor fibroblast landscape, which involved an expansion of IFN-licensed CAFs. This distinctive CAF population is marked by strong responses to IFN signaling, either by demonstrating increased MHC molecules and enhanced APM system, or by promoting T-cell infiltration via CXCR3 [267]. Despite "heating" the TIME, the strategy of targeting TGF-β seems unable to reverse the established CAF phenotype [266]. Indeed, the effective inhibition of TGF-β1 downstream mediators, such as the CXCL12/CXCR4 axis and NOX4, holds great potential for overcoming T-cell exclusion while maintaining a remarkable safety profile. TAMs are another promoter of fibrillar collagen by stimulating CAFs [268]. The coexistence of tumor-specific $SPP1^+$ TAMs and CAFs has been identified at the tumor boundary in the TIME of CRC and HCC. Blocking or specifically deleting SPP1 in the macrophages of murine models disrupts the desmoplastic microenvironment, leading to reduced CAF infiltration and enhanced cytotoxic T-cell infiltration [269, 270]. A study of PDAC revealed a feedback mechanism between macrophages and fibroblasts. Fibroblast-secreted IL-33 stimulates macrophages to produce CXCL3, which may act as an IL-33 imitator that targeted CXCR2 in stromal fibroblasts, thereby promoting CAF transition and collagen III generation [271].

Evidence is prominent for trials combining therapeutic approaches to address both the tumor stroma and malignant cells, which may unleash further advantages (Table 2). Recently, prior administration of FAP-CAR T cells has shown improved efficacy for subsequent tumor antigen (mesothelin)-targeted CAR T cells or anti-PD-1 antibody therapy [247]. On the one hand, the removal of FAP^+ CAFs disrupts the dense matrix and stromal border around tumor clusters, thereby facilitating the trafficking of cytotoxic effector cells and their direct communication with cancer cells. On the other hand, FAP-CAR T cells encourage T cell infiltration by inhibiting the CXCL12/CXCR4 axis and reducing chemokines (such as CCL3/4/5) which suppress the recruitment of immunosuppressive myeloid cells within the TIME. However, FAP is expressed in certain healthy tissues; thus, complete ablation of FAP is impractical and can potentially cause toxicity such as cachexia and anemia [272]. As a study on BC suggested, different FAP^+ stromal cells may exhibit dissimilar functions, phenotypes, and distributions; therefore, FAP-based treatments require careful assessments [273]. To circumvent these puzzles, "reprogramming" CAFs may be a prospective alternative. One possible solution is to administer Vitamin D agonists to restore quiescent normal fibroblasts, which is currently under investigation in an ongoing clinical trial (NCT03520790) [274]. Furthermore, blocking CTLA-4 on $CD8^+$ T cells counteracted CAF-mediated T-cell exclusion without affecting CAF levels [33]. The build-up of CAF-induced solid stress highlights formidable challenges for CTL infiltration and immunotherapies that depend on either endogenous or adoptively transferred T cells. Losartan has previously been demonstrated to alleviate solid stress by reducing collagen levels while increasing normalized HEVs, resulting in effective T-cell entry [247].

Overall, rather than intrinsic disorders of $CD8^+$ T cells, the transformed stromal microarchitecture that favors peritumoral retention of immune cells may play a more critical role for immune-excluded tumors. Further research is required to elucidate how specific therapeutic approaches manipulate ECM distribution and T-cell infiltration. Analyzing stromal heterogeneity across cancer types and treatments from a high-dimensional perspective is essential to comprehending the diverse roles of cell components within the stroma and identifying novel treatment strategies.

Metabolic disorders shaping the TIME

Transformation of the metabolic landscape in the TIME has been deemed as an established hallmark of cancer [275]. Malignant cells and tumor-residing cells

Table 2 Clinical trials targeting CAFs in different tumor indications

Categorization	Tumor Indications	Clinicaltrials.gov Identifier	Treatment Arms	Current Status
CAF Normalization	Metastatic Pancreatic Cancer	NCT03520790	Paricalcitol (Vitamin D receptor agonist) Gemcitabine Nab-paclitaxel	Phase 1/2
	Pancreatic Cancer	NCT03331562	Paricalcitol (Vitamin D receptor agonist) Pembrolizumab	Phase 2
	Squamous Cell Carcinoma of Head and Neck	NCT05323656	Setanaxib (GKT137831, NOX4 inhibitor) Pembrolizumab	Phase 2
Targeting Downstream Effectors	Metastatic Pancreatic Cancer	NCT02734160	Galunisertib (LY2157299, TGF-β Receptor I Kinase Inhibitor) Durvalumab	Phase 1
	Urothelial Carcer	NCT04064190	Vactosertib (TGF-β Inhibitor) Durvalumab	Phase 2
	Breast Cancer; Lung Cancer; Hepatocellular Cancer; Colorectal Cancer; Pancreatic Cancer; Renal Cancer	NCT02947165	NIS793 (anti-TGF-β monoclonal antibody) Spartalizumab	Phase 1
	Thymic Cancer; Thymoma	NCT04417660	Bintrafusp Alfa (M7824, bifunctional fusion protein targeting PD-L1 and TGF-β)	Phase 2
	Urothelial Cancer	NCT04501094	Bintrafusp alfa (M7824, bifunctional fusion protein targeting PD-L1 and TGF-β)	Phase 2
	NSCLC	NCT03631706	Bintrafusp alfa (M7824, bifunctional fusion protein targeting PD-L1 and TGF-β)	Phase 3
	Solid Tumors	NCT04291079	SRK-181 (anti-latent TGFβ1 monoclonal antibody) Anti-PD-(L)1 antibody therapy	Phase 1
	NSCLC	NCT04515979	Vactosertib (TEW-7197, TGFβ1 inhibitor) Pembrolizumab	Phase 2
	Pancreatic Cancer	NCT02907099	BL-8040 (CXCR4 antagonist) Pembrolizumab	Phase 2
	Metastatic Pancreatic Adenocarcinoma	NCT02826486	BL-8040 (CXCR4 antagonist) Pembrolizumab Chemotherapy of Onivyde	Phase 2
	Pancreatic Adenocarcinoma; Metastatic Ovarian Serous Adenocarcinoma; Colorectal Cancer Metastatic	NCT02179970	Plerixafor (CXCR4 antagonist)	Phase 1
	Solid Tumors	NCT02754141	BMS-986,179 (antibody inhibiting CD73 enzymatic activity) Nivolumab rHuPH20	Phase 1/2
	Renal Cell Carcer	NCT05501054	Ciforadenant (adenosine A2a receptor antagonist) Ipilimumab Nivolumab	Phase 1/2
	Renal Cell Cancer; Metastatic Castration Resistant Prostate Cancer	NCT02655822	Ciforadenant (adenosine A2a receptor antagonist) Atezolizumab	Phase 1

Table 2 (continued)

Categorization	Tumor Indications	Clinicaltrials.gov Identifier	Treatment Arms	Current Status
Targeting CAF-derived ECM Proteins	NSCLC	NCT02346370	PEGylated Recombinant Human Hyaluronidase (PEGPH20, to degrade major ECM component hyaluronan) Docetaxel	Phase 1
	Pancreatic Cancer	NCT03634332	PEGylated Recombinant Human Hyaluronidase (PEGPH20, to degrade major ECM component hyaluronan) Pembrolizumab	Phase 2
	Lymphoma; Melanoma; Renal Cell Carcinoma	NCT00001683	COL-3 (NSC-683551, matrix metalloproteinase inhibitor)	Phase 1
Depleting CAF	Breast Cancer; Cancer of Head and Neck	NCT02627274	FAP-IL2r (RO6874281, immunocytokine consisting of interleukin 2 variant targeting FAP) Trastuzumab Cetuximab	Phase 1
	Metastatic Melanoma	NCT03875079	FAP-IL2r (RO6874281, immunocytokine consisting of interleukin 2 variant targeting FAP) Pembrolizumab	Phase 1
	Renal Cell Carcinoma	NCT03063762	FAP-IL2r (RO6874281, immunocytokine consisting of interleukin 2 variant targeting FAP) Atezolizumab Bevacizumab	Phase 1
	Metastatic Colorectal Cancer	NCT04826003	FAP-4-1BBL (RO7122290, FAP targeted 4-1BB Ligand) Cibisatamab Obinutuzumab	Phase 1/2
Inhibiting CAF Activation	Ovarian Cancer	NCT01778803	Defactinib (VS-6063, Focal Adhesion Kinase Inhibitor) Paclitaxel	Phase 1
	Pancreatic Cancer	NCT02546531	Defactinib (VS-6063, Focal Adhesion Kinase Inhibitor) Pembrolizumab Gemcitabine	Phase 1

dynamically engage in spatial and temporal cooperations. The interconnected network for resource acquisition and exchange in TIME necessitates a holistic outlook. Tumor cells proactively exploit and manipulate local metabolite availability, establishing their dominance for energy and nutrients over non-cancerous cells [276]. For instance, under the hostile hypoxic condition of CRC, the upregulation of stanninocalcin 2, a glycoprotein hormone involved in glutamine or glucose deprivation, has been implicated in the preparation of tumor cells adapted to metabolic shifts, thereby promoting tumor progression [277]. TILs are subjected to the incurred metabolic stress, which drives the derangements of their metabolic programs. In CRC, with the combined application of multiplexed ion beam imaging by time of flight (MIBI-TOF) and antibody-based single-cell metabolic regulatory profiling (scMEP), Hartmann et al. uncovered a $CD8^+$ T cell subset with CD39 and PD-1 expression that was metabolically repressed and excluded from the tumor-immune boundary, indicating niche-driven modulation of immune cell distribution and functionality [278]. Indeed, high levels of hypoxia, lactate, acidification, as well as deficiency of essential amino acids, all modify the immunometabolism of immune cells and have been appreciated as determinants of a diminished intra-tumoral T cell pool [279].

Hypoxia driving multifaceted TIME perturbations

Solid stress and hypoxic state of the TIME are interdependent. Specifically, insufficient oxygen is a common characteristic across a spectrum of solid tumors, primarily because of limited oxygen delivery caused by aberrant neovascularization and robust matrix desmoplasia, both of which are indicative of high mechanical compression. Proliferative malignant cells outstrip blood supply and compete with neighboring immune cells for oxygen and nutrients [280]. It should be noted that the oxygen distribution within tumors is spatially heterogeneous and influenced by their proximity to blood vessels [281]. Recent studies on immunometabolism have suggested that oxygen tension is a tightly linked parameter of the tumor immune landscape. The hypoxic TIME undergoes drastic metabolic perturbations, leading to a metabolic barrier to efficient tumor elimination. According to Sugiura et al., immune-desert tumors employ metabolic adaptations against efficient T cell functionality and proliferation [282]. As a proof-of-concept, a preclinical investigation into prostate cancer revealed that hypoxic

niches displayed resistance to CTL infiltration, even in the presence of CTLA-4 and PD-1 dual-blockade, unless a hypoxia-reliving therapeutic approach was applied [283]. Intriguingly, supplemental oxygen can markedly alleviate the hypoxic conditions of TILs and promote T cell infiltration in murine models [284].

Importantly, multifaceted hypoxic signatures are associated with the immune-cold subtypes of various tumors [285, 286]. Specifically, glioblastoma is notorious for its immunologically "desert" TIME, where hypoxic niches, located distal to the incompetent vasculature, are found to entrap TAMs and CTLs and subsequently reprogram them into immunosuppressive state [287]. Mechanistically, TAM-derived CCL8 and IL-1β are essential hypoxic-niche factors to further attract and retain more cancer-killing cells, creating a vicious circle of this distinct temporospatial pattern. As hypoxia level rises, both in vivo and in vitro studies have reported impaired IFN-γ-dependent MHC-I expression, which is reversible once the oxygen-level is restored [288, 289]. Additionally, the hypoxia-induced ecto-nucleotidase CD39 coordinates with CD73 on Tregs to digest ATP and ADP into adenosine [290], which suppresses CTLs and induces a T cell exhaustion program [291]. The hypoxic TIME may also disadvantage T cells into terminal-exhausted state by driving mitochondrial stress and further facilitates the "quantitative cold" immunophenotype. ZipSeq, a spatial transcriptomic technique, maps exhaustion-related gene expression patterns and shows enrichment in hypoxic areas within the TIME [292].

Moreover, hypoxia is responsible for aggravating ECM remodeling by inducing enzymes, such as lysyl oxidases and collagen prolyl 4-hydroxylase, ultimately excluding T cells [293]. Previous studies have also demonstrated that long-term hypoxia in tissues can enhance TGF-β signaling. Indeed, the downstream HIFs and TGF-β are reciprocally induced, contributing to the disorganized ECM structure that further exacerbates solid stress and hypoxia [38, 294]. As revealed in colorectal adenocarcinoma, a string of signaling activation is involved in hypoxia-induced biological functions, including WNT, HIF-1, and ECM-related pathways. These signaling pathways collectively remodel the TIME in colorectal adenocarcinoma, which tends to present an excluded immunophenotype [295].

Aerobic glycolysis inducing lactate accumulation and acidosis in the TIME (Fig. 5)

The hypoxic TIME demonstrates a shift towards glycolytic metabolism owing to the potent activation of HIF-related genes. Metabolic reprogramming promotes aerobic glycolysis, also termed as "Warburg effect", which is a well-recognized hallmark of cancer [296, 297]. Regardless of the oxygen level, tumor cells prioritize glycolysis as an energy resource and readily convert glucose into large amount of lactate [298]. In this context, HIF-1α induces the overexpression of monocarboxylate transporter 4 (MCT4) on tumor cells, which facilitates the draining of lactate into the TIME so as to maintain intracellular PH homeostasis. In lung adenocarcinoma, the serine/ threonine kinase STK11 (also called LKB1) is a frequently mutated tumor suppressor gene that has been identified as the main driver of the inert immune-cold phenotype, despite the presence of a paradoxically high TMB due to LKB1 deficiency [299]. LKB1-mutant lung adenocarcinoma presents drastic metabolic alterations with elevated MCT4 expression, enhanced MCT4-dependent lactate secretion polarizes macrophages into the immunosuppressive M2 subtype and hampers T cell function as a consequence [300, 301]. In this sense, targeting the MCT4 lactate transporter offers a therapeutic route for overcoming the "cold" immune phenotype with restored CTL frequency and activity.

Immunometabolism studies have revealed that antitumor immune cells share comparable nutrients as cancerous cells, giving rise to a competitive dynamic between them. Similarly, T cells display glycolysis characteristics that sustain proliferation and increase fitness under extremely oxygen-deficient conditions [296]. Because of the metabolic tug-of-war during rapid cancer progression, T cells are unable to eliminate their targets with full potential. Notably, the level of glucose transporter type 1 (GLUT1) in tumors is inversely associated with $CD8^+$ T cell infiltration and survival in squamous cell carcinoma [302]. However, T cells in certain cancers demonstrate an intrinsic impairment in glycolysis. In human ccRCC, T cells may downregulate GAPDH, which leads to insufficient glucose uptake and use, even under the nutrient-replete conditions [303]. In this case, IFN-γ production can be compromised given GAPDH is exactly engaged in the posttranscriptional regulatory process of this proinflammatory cytokine [304]. Moreover, glucose deprivation of T cells may also induce the anergic exhausted state, leading to the "quantitative cold" TIME with impaired functional properties [305]. Neutralizing tumor acidity has been shown to improve response to ICB therapies [306]. Nevertheless, immunosuppressive Tregs can be invigorated in this therapeutic context due to lactate-induced PD-1 expression on Tregs, potentially compromising the efficacy of immunotherapies [307]. Glucose deprivation may not be a universal characteristic for malignant tumors. In a study of melanoma, neither a deficiency in GLUT1 expression nor an inability of CTLs to uptake glucose from the TIME was observed. Rather, $CD8^+$ T cells demonstrate constrained glucose metabolism owing to the impaired activity of enolase, a critical enzyme in the glycolytic pathway. ICB therapies retard melanoma progression by increasing CTL infiltration

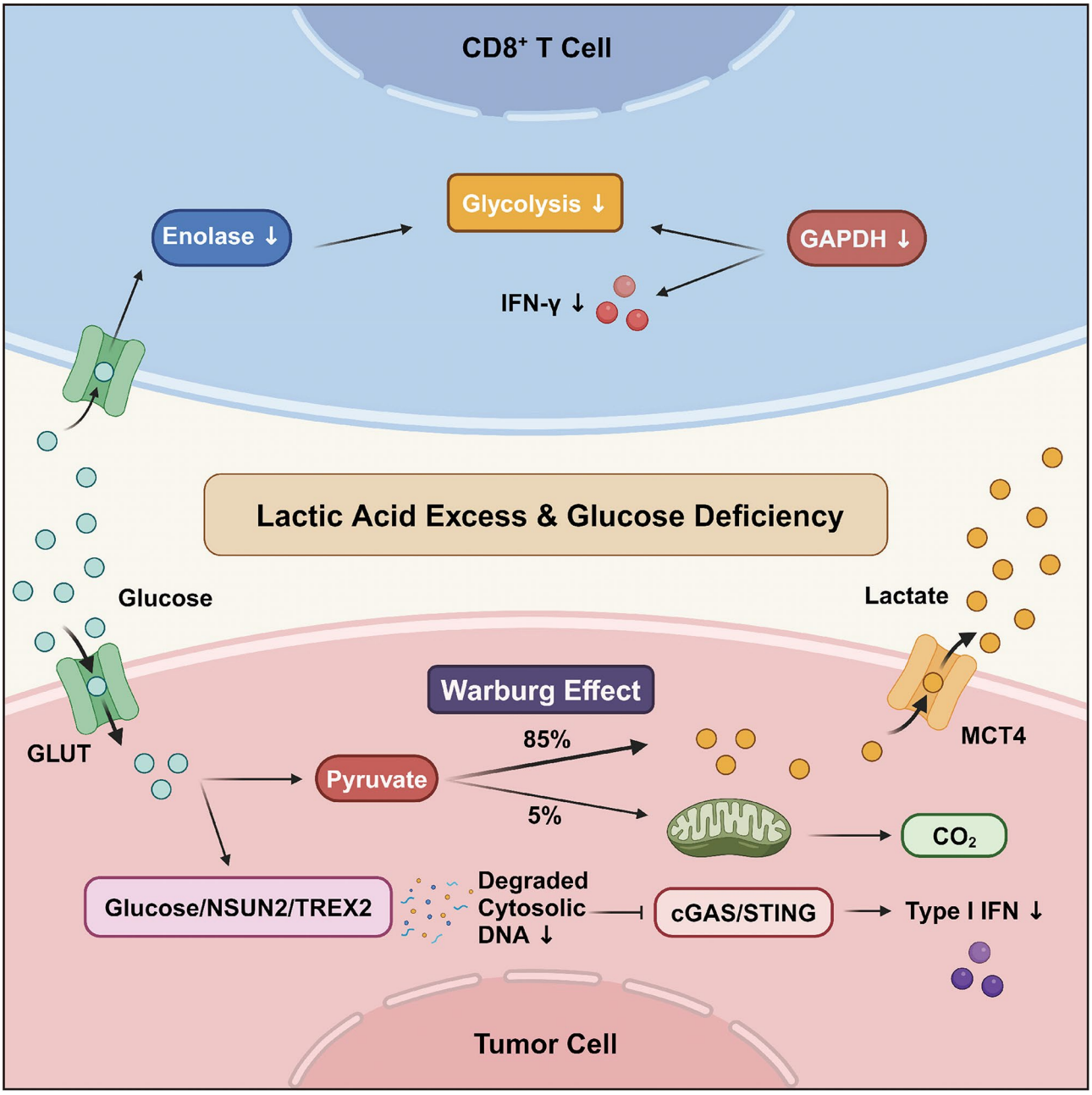

Fig. 5 Exacerbated Glucose Competition between Tumor and $CD8^+$ T cells in the Hypoxic TIME. Immune-noninflamed (immune-excluded and -desert phenotype) tumors undergo metabolic adaptations to compete for more oxygen and glucose against $CD8^+$ T cells. Mechanistically, immune-noninflamed tumors enhance glucose uptake by upregulating the glucose transporter GLUT. In the hypoxic TIME, this metabolic competition is further intensified by the Warburg effect, as tumor cells prioritize aerobic glycolysis, converting glucose into a substantial amount of lactate. Accordingly, the lactate transporter MCT4 is upregulated in the hypoxic condition, which facilitates the efflux of lactate and subsequently promotes TIME acidification. Glucose also serves as signaling molecule, as the glucose/NSUN2/TREX2 axis can shut off the cGAS/STING pathway, which is crucial for T cell recognition and infiltration in the immune-inflamed TIME. Conversely, $CD8^+$ T cells in immune-noninflamed tumors demonstrate constrained glucose metabolism characterized by insufficient uptake and impaired activity of key enzymes involved in the glycolytic pathway, such as enolase and GAPDH. Furthermore, the reduced activity of GAPDH can compromise the generation of proinflammatory cytokine IFN-γ at the posttranscriptional level. *Abbreviations*: GLUT: Glucose Transporter Type 1; MCT4: Monocarboxylate Transporter 4; NSUN2: NOP2/Sun RNA Methyltransferase 2; TREX2: Three Prime Repair Exonuclease 2; GAPDH: Glyceraldehyde-3-phosphate Dehydrogenase

with restored enolase activity, which was observed either in the recently activated CTLs or in the newly tumor-infiltrating T cells instead of reactivating enolase in the pre-existing CTLs [308, 309]. Moreover, in experimental settings, an adequate amount of ^{18}F-fluorodeoxyglucose is generally provided, which does not accurately reflect glucose availability within the TIME or the proficiency of glucose uptake by cells.

Admittedly, most investigations deem aberrant glucose metabolism as the key mediator of the TIME immune status. However, a recent study has suggested that glucose can directly disengage the immune response as a signaling molecule [310]. The glucose/NSUN2/TREX2 axis restricts cytosolic dsDNA accumulation, which shuts off cGAS/STING signaling for apoptosis, and consequently thwarts both $CD8^+$ T cell recognition and infiltration. Targeting this axis offers promising insights to resetting immune-cold tumors with aberrant glucose signaling into the inflamed tumors. This novel paradigm can precondition refractory immune-cold tumors such as prostate cancer and luminal subtype BC for enhanced efficacy in subsequent immunotherapies.

Amino acids deprivation (Fig. 6)

Compared to glucose that has garnered significant attention, researchers have only recently begun to delve into the impact of amino acids on tumor immune compartments. In a study on colon cancer, Rathmell et al. examined the metabolic features of various cell components in the TIME and discovered that $CD8^+$ T cells were not deficient in glucose, in contrast, cancer cells outcompeted in glutamine consumption four-fold higher than that of $CD8^+$ T cells [311]. In vivo metabolic tracer experiments revealed that TIME-residing cells do not consume nutrients proportionately. Specifically, cancer cells take up the lion's share of glutamine, whereas cells of the myeloid lineage, such as macrophages, are the primary consumers of glucose [311]. Furthermore, cancer cells may benefit from the cross-feeding of amino acids by other tumor-residing cells [312]. In ovarian cancer, CAF-secreted glutamine via solute carrier family 7 member 5 can be leveraged by cancer cells to fuel progression [313]. Therefore, one can envision that the unbalanced amino acids partitioning between cancer and immune cells would favor malignant cells while shaping the different immunophenotypes.

As with glucose, there may be competition for glutamine between cancer and immune cells, creating a scenario in which tumor cells outperform the uptake of local glutamine. T cells rely heavily on extracellular glutamine availability rather than de novo synthesis upon activation [314]. Accumulating evidence has indicated that glutamine plays immunomodulatory role for TILs, as a higher rate of glutamine use correlates with a lower apoptosis rate of themselves [286]. Investigations on human basal-like BC have proposed an inverse relationship between tumor glutamine metabolism and T cell cytotoxicity markers [315]. Furthermore, glutamine starvation hampers nucleotide synthesis and cytokine production, thereby impairing T cell activation and proliferation [314]. Genetic ablation of glutaminase (GLS), a critical enzyme that boosts conversion into glutamate, in tumor cells could lead to increased glutamine concentration and improved T-cell infiltration within tumor nests. Therefore, glutamine use by tumor cells is a potential immunoregulatory metabolic checkpoint that alters the characteristics of TILs. Under hypoxic conditions, HIF can equip cancer cells with the upregulated EPH receptor B2 for the uptake and accumulation of glutamine [286]. Additionally, the major glutamine transporter, ASC amino-acid transporter 2 (ASCT2), is overexpressed in cancers, such as melanoma and prostate cancer [316]. For TNBC that is specifically "glutamine-addicted", tumor cells exhibit elevated levels of ASCT2 as well as GLS [317]. Likewise, studies have found in hypoxic CRC, HIF-1 activates the promoter of GLS-1, thereby accelerating the rate-limiting step in glutaminolysis [318]. In addition to cancer cells, the anabolic program within CAFs can also deprive TILs of glucose and glutamine. Especially for immune-excluded tumors, TGF-β coordinates CAFs towards robust production of matrix proteins, which are highly enriched with proline and glycine [319]. To this end, CAFs increase the consumption of these raw materials, facilitating the synthesis of glycine from glucose via the serine biosynthetic pathway, and generating proline from glutamine. Indeed, evidence suggests that CAFs are sensitive to glutamine concentration within the TIME, favoring their transfer towards glutamine-high regions [320]. The glutamine distribution gradient increases towards the peripheral area in BC, which may explain why CAFs tend to retain in the peripheral stroma and induce immune-exclusion [320]. In turn, CAFs direct tumor cells towards these glutamine-enriched territories, contributing to tumor progression and metastasis [294]. Additionally, elevated tumor niche stiffness was found to mechanoactivate glycolysis and glutamine metabolism in cancer cells and CAFs via the YAP/TAZ pathway, creating a vicious circle of tumoricidal cell immunometabolism [321]. Activated T cells increase glutamine intake and metabolism to support various biological processes, including mitochondrial anaplerosis, nucleotide synthesis, amino acid generation, and redox balance, without which T cell-mediated immune responses are impaired [322].

For TNBC that are characterized by glutamine-addicted metabolism, targeting cancer metabolic reprogramming to reverse the tumor "glutamine steal" phenomenon, while sparing anti-tumor T cells, may hold therapeutic insights. In a TNBC murine model

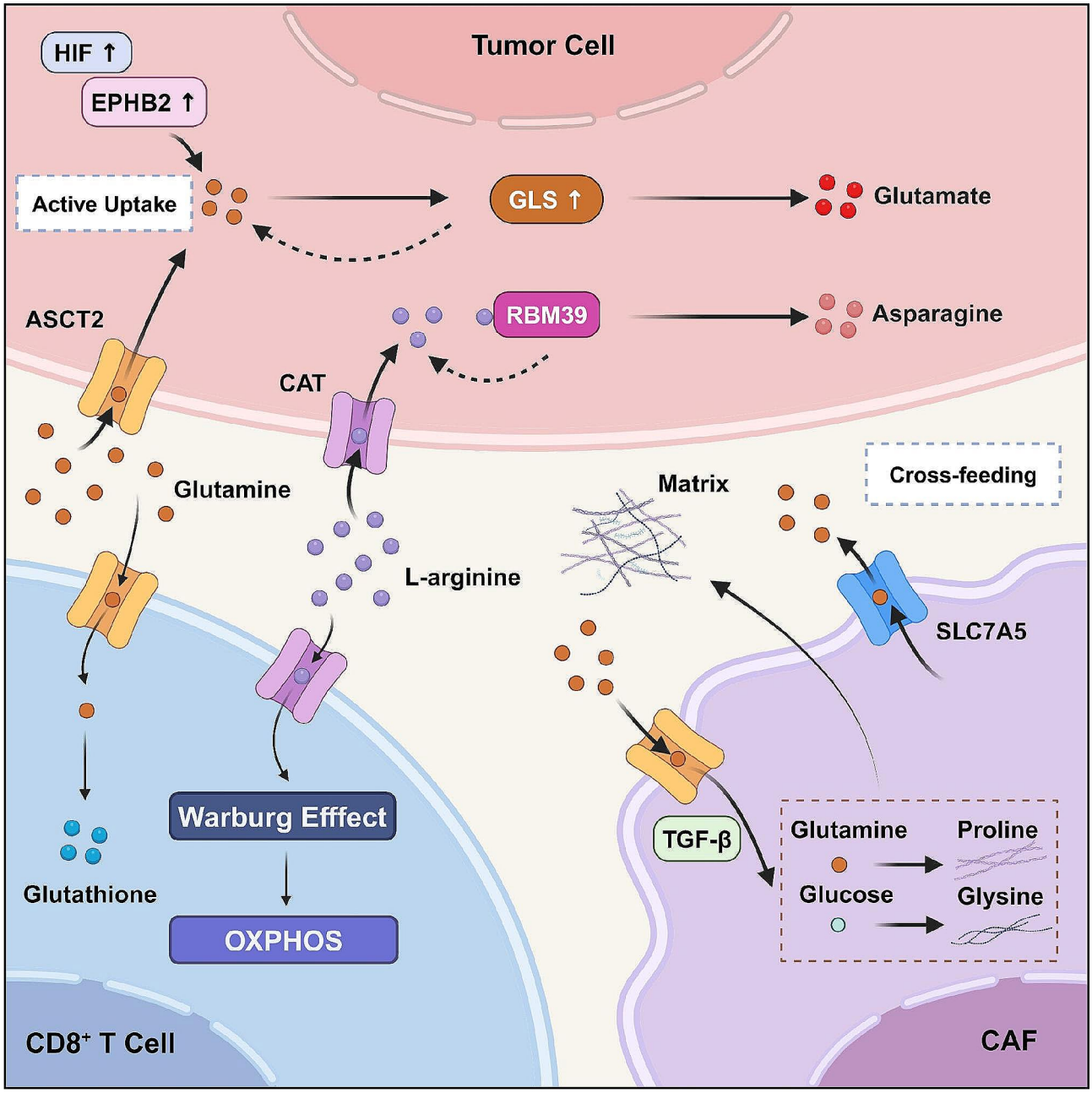

Fig. 6 Tumor Cells Outcompete CD8$^+$ T Cells in Essential Amino Acid Consumption to Promote Immune-excluded or -desert TIME. In immune-noninflamed (immune-excluded and -desert phenotype) TIME, tumors acquire large amount of glutamine either by overexpressing glutamine transporters or by cross-feeding from other cells such as CAFs. The former mechanism involves upregulated GLS in glutaminolysis, which in turn accelerates glutamine uptake by tumors. In the hypoxic TIME, HIF can equip tumor cells with upregulated EPHB2 and activate GLS promoter to accumulate glutamine. Moreover, CAFs can secrete glutamine via SLC7A5, which can be utilized by tumor cells to their advantage, especially for immune-excluded ones. Comparatively, CD8$^+$ T cells demonstrate limited access for glutamine in immune-noninflamed tumors, the consequent deficiency of antioxidant glutathione has been associated with reduced T-cell density and impaired function. L-arginine is critical for CD8$^+$ T cells to shift from the Warburg effect to OXPHOS, however, the lion's share of extracellular L-arginine is taken up by immune-noninflamed tumors through CAT. Then, L-arginine binds with RBM39 for further asparagine synthesis, which in turn enhances arginine uptake. Furthermore, CAFs, the predominant component of the immune-excluded TIME, are significantly involved in depriving CD8$^+$ T cells of essential amino acids as well. Mechanistically, CAFs favor migrating towards glutamine-high areas and subsequently provide guidance for tumor cells. Meanwhile, the upregulated TGF-β signaling coordinates CAFs to produce matrix proteins that are enriched with proline and glycine, which are transformed from glutamine and glucose respectively, thereby aggravating immune-exclusion as a result. *Abbreviations*: CAFs: Cancer-associated Fibroblasts; GLS: Glutaminase; HIF: Hypoxia-inducible Factor; EPHB2: EPH Receptor B2; SLC7A5: Solute Carrier Family 7 Member 5; OXPHOS: Oxidative Phosphorylation; CAT: Cationic Amino Acid Transporter; RBM39: RNA-binding Motif Protein 39

with specific GLS loss on tumor cells, studies revealed an elevated glutamine concentration in the tumor stroma that promoted the synthesis of glutathione, a major cellular antioxidant, in T cells to improve intra-tumoral $CD8^+$ T cell density and functionality. Moreover, in poorly immunogenic and "non-inflamed" EGFR-driven lung cancer, oral administration of a specific glutamine antagonist against cancer cells, JHU083, facilitated the enrichment of $CD8^+$ T cells [323, 324]. JHU083 is capable of converting immunosuppressive MDSCs and TAMs into tumor-destroying proinflammatory phenotypes [325]. Furthermore, pharmacological blockade of ASCT2 with V-9302 preferentially inhibits glutamine use in cancer cells, which upregulates the immune checkpoint factor PD-L1 by impairing the activity of Sarco/ER Ca^{2+}-ATPase (SERCA). Therefore, co-treatment with glutamine depletion and anti-PD-L1 antibody, therefore, is feasible and represents a promising strategy with synergistic anti-tumor effects and increased T- cell infiltration [326]. Taken together, an integrated understanding of glutamine metabolism in the TIME is of utmost importance as it provides crucial pathways that could be targeted by novel strategies. Indeed, it has the potential to yield a two-pronged attack that bolsters tumoricidal immune responses, while concurrently crippling tumor metabolism.

In addition to glutamine, L-arginine is known to regulate glycolysis and mitochondrial activity by interacting with transcriptional regulators that are essential for the function of TILs. Studies have indicated that intracellular L-arginine can prompt a metabolic transition from glycolysis to oxidative phosphorylation (OXPHOS) in activated T cells, thereby counteracting the Warburg effect and decreasing lactate production in the TIME [327]. One underlying mechanism is that elevated L-arginine may upregulate the serine biosynthesis pathway, which can facilitate OXPHOS [327]. Tumors actively take up arginine via cationic amino acid transporters, as documented in the context of hepatocellular carcinoma, which bind to the RNA-binding motif protein 39 (RBM39) for further metabolic reprogramming. Importantly, RBM39-mediated elevation of asparagine synthesis promotes arginine uptake, thereby forming a positive feedback loop to sustain arginine accumulation and oncogenic metabolism [328]. Additionally, arginase 1-expressing myeloid cells, which are prevalent in immune-cold tumors, primarily deplete T cells of arginine, resulting in the blockade of TCR expression and anti-tumor response [329]. PDAC is characterized by abundant infiltration of myeloid cells, leading to uncontrolled metabolism of L-arginine by arginase 1 and iNOS activity. Consequently, the generation of reactive nitrogen species establishes a chemical barrier that shields tumor cells from CTL recognition and entrance into the tumor core [330]. Because locally restoring the intra-tumoral L-arginine concentration can be challenging, Canale et al. devised an innovative engineered bacterium that can colonize tumor nests and convert the metabolic waste product ammonia to L-arginine, resulting in an increased frequency of TILs and additive effects with ICB treatments [331].

Metabolism-targeted interventions

Targeting dysregulated metabolic alterations in the TIME serves as an intriguing avenue for eliminating tumors. Therapeutic approaches involve targeting cancer cell metabolism to transform the TIME into a more conducive one for T cell efficacy. The GLS inhibitor CB-830 is currently undergoing phase 1 clinical trials (NCT02071862, NCT03875313, and NCT02861300) with inspiring outcomes. Glycolysis inhibitors, such as 2-Deoxyglucose, have garnered increasing popularity for the treatment of CRC at the preclinical stage [332]. Because TNBC also displays high glycolytic rates, the GLUT1 inhibitor BAY-876 has shown significant effectiveness in counteracting tumor proliferation. However, targeting glycolysis is not universally effective, as glycolysis serves as a crucial source of energy over various cell populations, and tumor cells also exhibit heterogeneity in carbohydrate metabolism. For instance, the infusion of 2-Deoxyglucose demonstrated an undesirable hypothalamus response in certain clinical trials due to its non-specificity [333]. To address this issue appropriately, a future strategy would be to introduce an agent that enhances the metabolic competitiveness of tumoricidal T cell populations within the TIME. Nutrient supplementation was also instrumental. Remarkably, a two-fold increase in intracellular L-arginine levels via oral administration has been demonstrated to promote the generation of central memory-like T cells in a murine model, which, when combined with adoptive T-cell therapy and immunotherapy, could potentially inflame the TIME and revive anti-tumor activity [327, 334]. A recent study outlined an interrelationship in which glutamine primes the tumor immunity of cDC1 [335]. Adequate intra-tumoral injection of glutamine licenses cDC1 for efficient antigen presentation, which has implications for augmenting $CD8^+$ T cell activation and overcoming ICB resistance.

However, randomized clinical trials on metabolism-targeted interventions have yielded unsatisfactory outcomes across the board [336]. Despite limited success in tumor elimination, these treatments may facilitate tumor progression by driving metabolic bypass, adaptation, differentiation, and therapeutic resistance. Li et al. discovered that, in response to glutamine starvation, the stress-induced transcription factor DNA damage induced transcript 3 is activated to promote glycolysis, thereby generating ATP to sustain tumor growth during metabolic stress [337]. In addition to this complexity,

metabolism-targeted drugs may also have "off-target" effects on non-cancerous cells. As such, these findings underscore the necessity of delineating the biological similarities and variations between target cancer cells and the other tumor-residing counterparts.

Conclusions

The TIME is a complex ecosystem composed of various cell types whose functionality and spatiality are typically hijacked to create a tumor-supportive and immune suppressive environment. ICB therapies have shown promise for patients with immune-inflamed tumors; however, such success is yet to be achieved for most immune-excluded or-desert tumors [338]. Exploring treatment strategies that can inflame the TIME serves as putative option for curing patients with cancer. The non-comparative phase II TONIC trial (NCT02499367) revealed that metastasized TNBC can be converted into an immune-inflamed state when preconditioned with chemotherapeutic agents, indicating the plasticity of immunophenotypes and the potential of priming non-inflamed tumors to favor immunotherapy [27]. Moreover, the majority of research conducted thus far has predominantly leveraged the pre-treatment state of the TIME to predict ICB responses, oversimplifying the fact that the TIME dynamically evolves alongside the tumor. Mariniello et al. demonstrated the superiority of sequential administration, in which chemotherapy is followed by PD-1 blockade, over a concomitant strategy [339]. This finding provides a strong rationale for delving deeper into the seemingly nuanced alterations in the TIME during treatments. To date, since holistic knowledge of the local TIME and overall tumor ecosystem is lacking, experiments and clinical trials on tumor immunophenotypes still yield certain contradictory outcomes.

The concept of personalized cancer immunotherapies has been ambitiously advocated. Precise immunophenotype-based stratification is integral to determine tailored therapeutic approaches in clinic settings, as highlighted by the IMvigor210 trial (NCT02951767 and NCT02108652), wherein a reduced panfibroblast TGF-response gene signature was linked with atezolizumab efficacy, but only restricted to immune-excluded tumors [33]. As prominent challenges persist in the accuracy and availability of TIME decoding technologies, their practical implementation has not been scaled up. Of note, our team comprehensively reviewed emergent multiomics technologies for deciphering the TIME in the context of TNBC [340]. Apart from conventional methods to evaluate the TIME, such as IHC and flow cytometry, revolutionary techniques are now available that approach towards a 3D-dimensioned standpoint and even integrate temporal analysis of the dynamic TIME [341]. Spatial transcriptomics combines high-resolution spatial architectures with single-cell RNA sequencing or single-nucleus RNA sequencing data, providing researchers with high throughput information across various biological samples [342]. Based on next-generation sequencing, spatially resolved transcriptomics technology has emerged in a timely manner with a unique position to delve into specific spatial structures in the TIME [160]. The integration of artificial intelligence has recently ushered in a new epoch to elucidate TIME patterns and establish feasible predictive models with improved objectivity, consistency, and comprehensiveness in clinical and investigational contexts [343–345]. Machine learning has facilitated the discovery of in-depth biomarkers and intercellular relationships within the TIME. For instance, skin cutaneous melanoma with an increased Banfield Raftery index (TIL cluster count) hinted towards favorable survival, whereas BC with a high Ball Hall index (TIL cluster extent) correlated with inferior prognosis [346, 347]. In laboratories, because cell line models cannot accurately recapitulate the crisscross communication network within the TIME, the advent of tumor organoids or patient-derived xenografts may fully capture the tumor ecosystem and better inform clinical trials [348]. Overall, by determining the spatiotemporal dynamics of the TIME, we can explore deeper into the contributors of different immunophenotypes and guide personalized precision medicine in the future.

Abbreviations

ICB	Immune checkpoint blockade
ACT	Adoptive cell therapy
CAR	Chimeric antigen receptor
PD-1	Programmed cell death 1
PD-L1	Programmed cell death ligand 1
CTLA-4	Cytotoxic T lymphocyte-associated antigen-4
TIME	Tumor immune microenvironment
ECM	Extracellular matrix
TILs	Tumor-infiltrating lymphocytes
CRC	Colorectal cancer
MSI	Microsatellite instability
sTILs	Stromal tumor-infiltrating lymphocytes
APM	Antigen presentation machinery
IFN	Interferon
IHC	Immunohistochemistry
3D	Three-dimensional
NSCLC	Non-small cell lung cancer
BC	Breast cancer
TNBC	Triple negative breast cancer
LS	Lynch syndrome
GI	Genomic instability
TMB	Tumor mutational burden
TMB-H	High tumor mutational burden
SCNA	Somatic copy number alteration
TCR	T-cell receptor
ccRCC	Clear cell renal cell carcinoma
GEA	Gastroesophageal adenocarcinoma
HER2	Human epidermal growth factor receptor-2
HIF-1	Hypoxia-inducible factor-1
DC	Dendritic cell
Tregs	Regulatory T cells
PDAC	Pancreatic ductal adenocarcinoma
TR	Tumor-reactive
APC	Antigen presenting cell

PAMP	Pathogen-associated molecular pattern
DAMP	Damage-associated molecular pattern
dLN	Draining lymph node
CLEC9A	C-type lectin domain containing 9 A
iDAMP	Inhibitory damage-associated molecular pattern
PGE2	Prostaglandin E2
PGRN	Progranulin
HEV	High endothelial venules
ANGPT2	Angiopoietin-2
AM	Adhesion molecule
VCAM-1	Vascular cell adhesion molecule 1
TET2	Teneleven translocation-2
cGAS	Cyclic GMP-AMP synthase
TLS	Tertiary lymphoid structure
HCC	Hepatocellular carcinoma
iCCA	Intrahepatic cholangiocarcinoma
I-TLS	Intra-tumoral tertiary lymphoid structure
P-TLS	Peritumoral tertiary lymphoid structure
MDSC	Myeloid-derived suppressor cell
TAM	Tumor-associated macrophage
M-MDSC	Monocytic myeloid-derived suppressor cell
PMN-MDSC	Polymorphonuclear myeloid-derived suppressor cell
ROS	Reactive oxygen species
iNOS	Inducible nitric oxide synthase
N-CCL2	Nitrify CCL2
NET	Neutrophil extracellular trap
Chi3l1	Chitinase-3-like 1
MCTC	Macrophage-coated tumor cluster
CAR-M	Chimeric antigen receptor macrophage
IDO	Indoleamine 2, 3-dioxygenase
CAF	Cancer-associated fibroblast
FAP	Fibroblast activation protein
POSTN	Periostin
TIDE	Tumor immune dysfunction and exclusion
PDPN	Podoplanin
SASP	Senescence associated secretory phenotype
NOX4	NADPH-oxidase-4
MIBI-TOF	Multiplexed ion beam imaging by time of flight
scMEP	Single-cell metabolic regulome profiling
MCT4	Monocarboxylate transporter 4
GLUT1	Glucose transporter type 1
GLS	Glutaminase
ASCT2	ASC amino-acid transporter 2
SERCA	Sarco/ER $Ca2^{+}$ -ATPase
OXPHOS	Oxidative phosphorylation
RBM39	RNA-binding motif protein 39

Acknowledgements
We thank BIORENDER (https://www.biorender.com/) for its figure creating assistance during the preparation of this manuscript.

Author contributions
All authors have read and approved the article. S.Z. and W.W. contributed to the literature collection and manuscript writing. L.S. and Y.Y. contributed to the review discussion and language editing. W.X. and C.N. participated in the design and review of the manuscript. S.Z. and W.W. contributed equally to this work.

Funding
This work was supported by the National Natural Science Foundation of China (Grant 82073151, 82273275), the Natural Science Foundation of Zhejiang Province (Grant LR19H160001, LGF21H160030), and the Fundamental Research Funds for the Central Universities (Grant 226-2024-00062).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 2 March 2024 / Accepted: 22 July 2024
Published online: 06 August 2024

References

1. Hoos A. Development of immuno-oncology drugs - from CTLA4 to PD1 to the next generations. Nat Rev Drug Discov. 2016;15(4):235–47.
2. Cappell KM, Kochenderfer JN. Long-term outcomes following CAR T cell therapy: what we know so far. Nat Rev Clin Oncol. 2023;20(6):359–71.
3. Wang Y, Zhang H, Liu C, Wang Z, Wu W, Zhang N, et al. Immune checkpoint modulators in cancer immunotherapy: recent advances and emerging concepts. J Hematol Oncol. 2022;15(1):111.
4. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Lao CD, et al. Five-year survival with combined Nivolumab and Ipilimumab in Advanced Melanoma. N Engl J Med. 2019;381(16):1535–46.
5. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, et al. Five-year survival outcomes for patients with advanced melanoma treated with pembrolizumab in KEYNOTE-001. Ann Oncol. 2019;30(4):582–8.
6. Garon EB, Hellmann MD, Rizvi NA, Carcereny E, Leighl NB, Ahn MJ, et al. Five-year overall survival for patients with Advanced non–small-cell lung Cancer treated with Pembrolizumab: results from the phase I KEYNOTE-001 study. J Clin Oncol. 2019;37(28):2518–27.
7. Jia Q, Wang A, Yuan Y, Zhu B, Long H. Heterogeneity of the tumor immune microenvironment and its clinical relevance. Exp Hematol Oncol. 2022;11(1):24.
8. Yuan Y, Li H, Pu W, Chen L, Guo D, Jiang H, et al. Cancer metabolism and tumor microenvironment: fostering each other? Sci China Life Sci. 2022;65(2):236–79.
9. Junttila MR, de Sauvage FJ. Influence of tumour micro-environment heterogeneity on therapeutic response. Nature. 2013;501(7467):346–54.
10. Gajewski TF. The next hurdle in Cancer Immunotherapy: overcoming the Non-t-cell-inflamed Tumor Microenvironment. Semin Oncol. 2015;42(4):663–71.
11. Kim SJ, Khadka D, Seo JH. Interplay between Solid Tumors and Tumor Microenvironment. Front Immunol. 2022;13:882718.
12. Taddei ML, Giannoni E, Comito G, Chiarugi P. Microenvironment and tumor cell plasticity: an easy way out. Cancer Lett. 2013;341(1):80–96.
13. Hegde PS, Chen DS. Top 10 challenges in Cancer Immunotherapy. Immunity. 2020;52(1):17–35.
14. Galon J, Bruni D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. Nat Rev Drug Discov. 2019;18(3):197–218.
15. Galon J, Angell HK, Bedognetti D, Marincola FM. The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures. Immunity. 2013;39(1):11–26.
16. Mlecnik B, Bindea G, Angell HK, Maby P, Angelova M, Tougeron D, et al. Integrative analyses of Colorectal Cancer Show Immunoscore is a stronger predictor of patient survival than microsatellite instability. Immunity. 2016;44(3):698–711.
17. Tang H, Wang Y, Chlewicki LK, Zhang Y, Guo J, Liang W, et al. Facilitating T cell infiltration in Tumor Microenvironment overcomes resistance to PD-L1 blockade. Cancer Cell. 2016;30(3):500.
18. Pages F, Mlecnik B, Marliot F, Bindea G, Ou FS, Bifulco C, et al. International validation of the consensus immunoscore for the classification of colon cancer: a prognostic and accuracy study. Lancet. 2018;391(10135):2128–39.
19. Angell H, Galon J. From the immune contexture to the immunoscore: the role of prognostic and predictive immune markers in cancer. Curr Opin Immunol. 2013;25(2):261–7.
20. Loibl S, Schneeweiss A, Huober J, Braun M, Rey J, Blohmer JU, et al. Neoadjuvant durvalumab improves survival in early triple-negative breast cancer independent of pathological complete response. Ann Oncol. 2022;33(11):1149–58.
21. Hegde PS, Karanikas V, Evers S. The where, the when, and the How of Immune Monitoring for Cancer immunotherapies in the era of checkpoint inhibition. Clin Cancer Res. 2016;22(8):1865–74.

22. Zhang J, Huang D, Saw PE, Song E. Turning cold tumors hot: from molecular mechanisms to clinical applications. Trends Immunol. 2022;43(7):523–45.
23. Adams S, Dieras V, Barrios CH, Winer EP, Schneeweiss A, Iwata H, et al. Patient-reported outcomes from the phase III IMpassion130 trial of atezolizumab plus nab-paclitaxel in metastatic triple-negative breast cancer. Ann Oncol. 2020;31(5):582–9.
24. Sweis RF, Spranger S, Bao R, Paner GP, Stadler WM, Steinberg G, et al. Molecular drivers of the Non-t-cell-inflamed Tumor Microenvironment in urothelial bladder Cancer. Cancer Immunol Res. 2016;4(7):563–8.
25. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature. 2017;541(7637):321–30.
26. Castiglioni A, Yang Y, Williams K, Gogineni A, Lane RS, Wang AW, et al. Combined PD-L1/TGFbeta blockade allows expansion and differentiation of stem cell-like CD8 T cells in immune excluded tumors. Nat Commun. 2023;14(1):4703.
27. Voorwerk L, Slagter M, Horlings HM, Sikorska K, van de Vijver KK, de Maaker M, et al. Immune induction strategies in metastatic triple-negative breast cancer to enhance the sensitivity to PD-1 blockade: the TONIC trial. Nat Med. 2019;25(6):920–8.
28. Desbois M, Wang Y. Cancer-associated fibroblasts: Key players in shaping the tumor immune microenvironment. Immunol Rev. 2021;302(1):241–58.
29. Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. Science. 2015;348(6230):74–80.
30. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature. 2013;500(7463):415–21.
31. Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, et al. Mutational landscape and significance across 12 major cancer types. Nature. 2013;502(7471):333–9.
32. Shukla SA, Rooney MS, Rajasagi M, Tiao G, Dixon PM, Lawrence MS, et al. Comprehensive analysis of cancer-associated somatic mutations in class I HLA genes. Nat Biotechnol. 2015;33(11):1152–8.
33. Mariathasan S, Turley SJ, Nickles D, Castiglioni A, Yuen K, Wang Y, et al. TGFbeta attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. Nature. 2018;554(7693):544–8.
34. Salmon H, Franciszkiewicz K, Damotte D, Dieu-Nosjean MC, Validire P, Trautmann A, et al. Matrix architecture defines the preferential localization and migration of T cells into the stroma of human lung tumors. J Clin Invest. 2012;122(3):899–910.
35. Kong X. Discovery of New Immune checkpoints: Family grows up. Adv Exp Med Biol. 2020;1248:61–82.
36. Mlynska A, Vaisnore R, Rafanavicius V, Jocys S, Janeiko J, Petrauskyte M, et al. A gene signature for immune subtyping of desert, excluded, and inflamed ovarian tumors. Am J Reprod Immunol. 2020;84(1):e13244.
37. Kather JN, Suarez-Carmona M, Charoentong P, Weis C-A, Hirsch D, Bankhead P, et al. Topography of cancer-associated immune cells in human solid tumors. Elife. 2018;7:e36967.
38. Desbois M, Udyavar AR, Ryner L, Kozlowski C, Guan Y, Durrbaum M, et al. Integrated digital pathology and transcriptome analysis identifies molecular mediators of T-cell exclusion in ovarian cancer. Nat Commun. 2020;11(1):5583.
39. Xin S, Liu X, Li Z, Sun X, Wang R, Zhang Z, et al. ScRNA-seq revealed an immunosuppression state and tumor microenvironment heterogeneity related to lymph node metastasis in prostate cancer. Exp Hematol Oncol. 2023;12(1):49.
40. Mlecnik B, Van den Eynde M, Bindea G, Church SE, Vasaturo A, Fredriksen T, et al. Comprehensive Intrametastatic Immune quantification and Major Impact of Immunoscore on Survival. J Natl Cancer Inst. 2018;110(1):97–108.
41. Song AH, Williams M, Williamson DFK, Chow SSL, Jaume G, Gao G, et al. Analysis of 3D pathology samples using weakly supervised AI. Cell. 2024;187(10):2502–20. e17.
42. Tanaka N, Kanatani S, Tomer R, Sahlgren C, Kronqvist P, Kaczynska D, et al. Whole-tissue biopsy phenotyping of three-dimensional tumours reveals patterns of cancer heterogeneity. Nat Biomed Eng. 2017;1(10):796–806.
43. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity. 2013;39(4):782–95.
44. Tavare R, Escuin-Ordinas H, Mok S, McCracken MN, Zettlitz KA, Salazar FB, et al. An effective Immuno-PET Imaging Method to monitor CD8-Dependent responses to Immunotherapy. Cancer Res. 2016;76(1):73–82.
45. Ali HR, Chlon L, Pharoah PD, Markowetz F, Caldas C. Patterns of Immune infiltration in breast Cancer and their clinical implications: a gene-expression-based retrospective study. PLoS Med. 2016;13(12):e1002194.
46. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust enumeration of cell subsets from tissue expression profiles. Nat Methods. 2015;12(5):453–7.
47. George J, Lim JS, Jang SJ, Cun Y, Ozretic L, Kong G, et al. Comprehensive genomic profiles of small cell lung cancer. Nature. 2015;524(7563):47–53.
48. Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang TH, et al. The Immune Landscape of Cancer. Immunity. 2018;48(4):812–30. e14.
49. Iglesia MD, Parker JS, Hoadley KA, Serody JS, Perou CM, Vincent BG. Genomic analysis of Immune Cell infiltrates across 11 Tumor types. J Natl Cancer Inst. 2016;108(11):djw144.
50. Gentles AJ, Newman AM, Liu CL, Bratman SV, Feng W, Kim D, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. Nat Med. 2015;21(8):938–45.
51. Aran D, Hu Z, Butte AJ. xCell: digitally portraying the tissue cellular heterogeneity landscape. Genome Biol. 2017;18(1):220.
52. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, et al. TIMER: a web server for Comprehensive Analysis of Tumor-infiltrating Immune cells. Cancer Res. 2017;77(21):e108–10.
53. Zappia L, Phipson B, Oshlack A. Exploring the single-cell RNA-seq analysis landscape with the scRNA-tools database. PLoS Comput Biol. 2018;14(6):e1006245.
54. Chen X, Sun YC, Church GM, Lee JH, Zador AM. Efficient in situ barcode sequencing using padlock probe-based BaristaSeq. Nucleic Acids Res. 2018;46(4):e22.
55. Jimenez-Sanchez A, Memon D, Pourpe S, Veeraraghavan H, Li Y, Vargas HA, et al. Heterogeneous Tumor-Immune Microenvironments among differentially growing metastases in an ovarian Cancer patient. Cell. 2017;170(5):927–38. e20.
56. Guo L, Kong D, Liu J, Zhan L, Luo L, Zheng W, et al. Breast cancer heterogeneity and its implication in personalized precision therapy. Exp Hematol Oncol. 2023;12(1):3.
57. Geurts V, Kok M. Immunotherapy for Metastatic Triple negative breast Cancer: current paradigm and future approaches. Curr Treat Options Oncol. 2023;24(6):628–43.
58. Hammerl D, Martens JWM, Timmermans M, Smid M, Trapman-Jansen AM, Foekens R, et al. Spatial immunophenotypes predict response to anti-PD1 treatment and capture distinct paths of T cell evasion in triple negative breast cancer. Nat Commun. 2021;12(1):5668.
59. Yi M, Niu M, Wu Y, Ge H, Jiao D, Zhu S, et al. Combination of oral STING agonist MSA-2 and anti-TGF-beta/PD-L1 bispecific antibody YM101: a novel immune cocktail therapy for non-inflamed tumors. J Hematol Oncol. 2022;15(1):142.
60. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity. 2013;39(1):1–10.
61. Chang K, Taggart MW, Reyes-Uribe L, Borras E, Riquelme E, Barnett RM, et al. Immune Profiling of Premalignant lesions in patients with Lynch Syndrome. JAMA Oncol. 2018;4(8):1085–92.
62. McGranahan N, Swanton C. Clonal heterogeneity and Tumor Evolution: past, Present, and the future. Cell. 2017;168(4):613–28.
63. Lemery S, Keegan P, Pazdur R. First FDA approval Agnostic of Cancer Site - when a Biomarker defines the indication. N Engl J Med. 2017;377(15):1409–12.
64. Chan TA, Yarchoan M, Jaffee E, Swanton C, Quezada SA, Stenzinger A, et al. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. Ann Oncol. 2019;30(1):44–56.
65. Gubin MM, Artyomov MN, Mardis ER, Schreiber RD. Tumor neoantigens: building a framework for personalized cancer immunotherapy. J Clin Invest. 2015;125(9):3413–21.
66. Wei SC, Duffy CR, Allison JP. Fundamental mechanisms of Immune Checkpoint Blockade Therapy. Cancer Discov. 2018;8(9):1069–86.
67. O'Meara TA, Tolaney SM. Tumor mutational burden as a predictor of immunotherapy response in breast cancer. Oncotarget. 2021;12(5):394–400.
68. Cristescu R, Mogg R, Ayers M, Albright A, Murphy E, Yearley J, et al. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. Science. 2018;362(6411):eaar3593.
69. Hellmann MD, Nathanson T, Rizvi H, Creelan BC, Sanchez-Vega F, Ahuja A, et al. Genomic features of response to Combination Immunotherapy in patients with Advanced Non-small-cell Lung Cancer. Cancer Cell. 2018;33(5):843–52. e4.
70. Rooney MS, Shukla SA, Wu CJ, Getz G, Hacohen N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell. 2015;160(1–2):48–61.

71. Liu D, Schilling B, Liu D, Sucker A, Livingstone E, Jerby-Arnon L, et al. Integrative molecular and clinical modeling of clinical outcomes to PD1 blockade in patients with metastatic melanoma. Nat Med. 2019;25(12):1916–27.
72. Alexandrov LB, Kim J, Haradhvala NJ, Huang MN, Tian Ng AW, Wu Y, et al. The repertoire of mutational signatures in human cancer. Nature. 2020;578(7793):94–101.
73. Davoli T, Uno H, Wooten EC, Elledge SJ. Tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy. Science. 2017;355(6322):eaaf8399.
74. Xie N, Shen G, Gao W, Huang Z, Huang C, Fu L. Neoantigens: promising targets for cancer therapy. Signal Transduct Target Ther. 2023;8(1):9.
75. Flippot R, Malouf GG, Su X, Khayat D, Spano JP. Oncogenic viruses: lessons learned using next-generation sequencing technologies. Eur J Cancer. 2016;61:61–8.
76. Smith CC, Beckermann KE, Bortone DS, De Cubas AA, Bixby LM, Lee SJ, et al. Endogenous retroviral signatures predict immunotherapy response in clear cell renal cell carcinoma. J Clin Invest. 2018;128(11):4804–20.
77. Panda A, de Cubas AA, Stein M, Riedlinger G, Kra J, Mayer T, et al. Endogenous retrovirus expression is associated with response to immune checkpoint blockade in clear cell renal cell carcinoma. JCI Insight. 2018;3(16):e121522.
78. Indini A, Massi D, Pirro M, Roila F, Grossi F, Sahebkar A, et al. Targeting inflamed and non-inflamed melanomas: biological background and clinical challenges. Semin Cancer Biol. 2022;86(Pt 2):477–90.
79. Derks S, de Klerk LK, Xu X, Fleitas T, Liu KX, Liu Y, et al. Characterizing diversity in the tumor-immune microenvironment of distinct subclasses of gastroesophageal adenocarcinomas. Ann Oncol. 2020;31(8):1011–20.
80. Woo SR, Corrales L, Gajewski TF. The STING pathway and the T cell-inflamed tumor microenvironment. Trends Immunol. 2015;36(4):250–6.
81. Fukai S, Nakajima S, Saito M, Saito K, Kase K, Nakano H, et al. Down-regulation of stimulator of interferon genes (STING) expression and CD8 + T-cell infiltration depending on HER2 heterogeneity in HER2-positive gastric cancer. Gastric Cancer. 2023;26(6):878–90.
82. Yi M, Niu M, Zhang J, Li S, Zhu S, Yan Y, et al. Combine and conquer: manganese synergizing anti-TGF-beta/PD-L1 bispecific antibody YM101 to overcome immunotherapy resistance in non-inflamed cancers. J Hematol Oncol. 2021;14(1):146.
83. Yi M, Wu Y, Niu M, Zhu S, Zhang J, Yan Y, et al. Anti-TGF-β/PD-L1 bispecific antibody promotes T cell infiltration and exhibits enhanced antitumor activity in triple-negative breast cancer. J Immunother Cancer. 2022;10(12):e005543.
84. Moore E, Clavijo PE, Davis R, Cash H, Van Waes C, Kim Y, et al. Established T cell-inflamed tumors rejected after adaptive resistance was reversed by combination STING activation and PD-1 pathway blockade. Cancer Immunol Res. 2016;4(12):1061–71.
85. Hu R, Han Q, Zhang J. STAT3: a key signaling molecule for converting cold to hot tumors. Cancer Lett. 2020;489:29–40.
86. Bernard S, Myers M, Fang WB, Zinda B, Smart C, Lambert D, et al. CXCL1 derived from mammary fibroblasts promotes progression of mammary lesions to Invasive Carcinoma through CXCR2 Dependent mechanisms. J Mammary Gland Biol. 2018;23(4):249–67.
87. Pinyol R, Sia D, Llovet JM. Immune Exclusion-Wnt/CTNNB1 class predicts resistance to immunotherapies in HCC. Clin Cancer Res. 2019;25(7):2021–3.
88. Takeuchi Y, Tanegashima T, Sato E, Irie T, Sai A, Itahashi K, et al. Highly immunogenic cancer cells require activation of the WNT pathway for immunological escape. Sci Immunol. 2021;6(65):eabc6424.
89. Pai SG, Carneiro BA, Mota JM, Costa R, Leite CA, Barroso-Sousa R, et al. Wnt/beta-catenin pathway: modulating anticancer immune response. J Hematol Oncol. 2017;10(1):101.
90. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. Nature. 2015;523(7559):231–5.
91. Parsons MJ, Tammela T, Dow LE. WNT as a driver and dependency in Cancer. Cancer Discov. 2021;11(10):2413–29.
92. Butti R, Gunasekaran VP, Kumar TVS, Banerjee P, Kundu GC. Breast cancer stem cells: Biology and therapeutic implications. Int J Biochem Cell Biol. 2019;107:38–52.
93. Peng W, Chen JQ, Liu C, Malu S, Creasy C, Tetzlaff MT, et al. Loss of PTEN promotes resistance to T cell-mediated immunotherapy. Cancer Discov. 2016;6(2):202–16.
94. Spranger S, Luke JJ, Bao R, Zha Y, Hernandez KM, Li Y, et al. Density of immunogenic antigens does not explain the presence or absence of the T-cell-inflamed tumor microenvironment in melanoma. Proc Natl Acad Sci U S A. 2016;113(48):E7759–68.
95. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer. 2012;12(4):298–306.
96. Yang B, Li X, Zhang W, Fan J, Zhou Y, Li W, et al. Spatial heterogeneity of infiltrating T cells in high-grade serous ovarian cancer revealed by multi-omics analysis. Cell Rep Med. 2022;3(12):100856.
97. Oliveira G, Stromhaug K, Klaeger S, Kula T, Frederick DT, Le PM, et al. Phenotype, specificity and avidity of antitumour CD8(+) T cells in melanoma. Nature. 2021;596(7870):119–25.
98. Zheng L, Qin S, Si W, Wang A, Xing B, Gao R, et al. Pan-cancer single-cell landscape of tumor-infiltrating T cells. Science. 2021;374(6574):abe6474.
99. Nunez NG, Tosello Boari J, Ramos RN, Richer W, Cagnard N, Anderfuhren CD, et al. Tumor invasion in draining lymph nodes is associated with Treg accumulation in breast cancer patients. Nat Commun. 2020;11(1):3272.
100. Ji AL, Rubin AJ, Thrane K, Jiang S, Reynolds DL, Meyers RM, et al. Multimodal Analysis of Composition and spatial Architecture in Human squamous cell carcinoma. Cell. 2020;182(6):1661–2.
101. Mandal R, Senbabaoglu Y, Desrichard A, Havel JJ, Dalin MG, Riaz N, et al. The head and neck cancer immune landscape and its immunotherapeutic implications. JCI Insight. 2016;1(17):e89829.
102. Zander R, Schauder D, Xin G, Nguyen C, Wu X, Zajac A, et al. CD4(+) T cell help is required for the formation of a cytolytic CD8(+) T cell subset that protects against chronic infection and Cancer. Immunity. 2019;51(6):1028–42. e4.
103. Scheper W, Kelderman S, Fanchi LF, Linnemann C, Bendle G, de Rooij MAJ, et al. Low and variable tumor reactivity of the intratumoral TCR repertoire in human cancers. Nat Med. 2019;25(1):89–94.
104. Simoni Y, Becht E, Fehlings M, Loh CY, Koo SL, Teng KWW, et al. Bystander CD8(+) T cells are abundant and phenotypically distinct in human tumour infiltrates. Nature. 2018;557(7706):575–9.
105. 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-Naive Melanoma patients and Expand significantly during Anti-PD-1 treatment. Clin Cancer Res. 2018;24(13):3036–45.
106. Attrill GH, Owen CN, Ahmed T, Vergara IA, Colebatch AJ, Conway JW, et al. Higher proportions of CD39 + tumor-resident cytotoxic T cells predict recurrence-free survival in patients with stage III melanoma treated with adjuvant immunotherapy. J Immunother Cancer. 2022;10(6):e004771.
107. Chen X, Zhao J, Yue S, Li Z, Duan X, Lin Y et al. An oncolytic virus delivering tumor-irrelevant bystander T cell epitopes induces anti-tumor immunity and potentiates cancer immunotherapy. Nat Cancer. 2024.
108. Tsuji T, Eng KH, Matsuzaki J, Battaglia S, Szender JB, Miliotto A, et al. Clonality and antigen-specific responses shape the prognostic effects of tumor-infiltrating T cells in ovarian cancer. Oncotarget. 2020;11(27):2669–83.
109. Lecuelle J, Boidot R, Mananet H, Derangère V, Albuisson J, Goussot V, et al. TCR clonality and Genomic Instability Signatures as prognostic biomarkers in high Grade Serous Ovarian Cancer. Cancers. 2021;13(17):4394.
110. Meng Z, Rodriguez Ehrenfried A, Tan CL, Steffens LK, Kehm H, Zens S, et al. Transcriptome-based identification of tumor-reactive and bystander CD8(+) T cell receptor clonotypes in human pancreatic cancer. Sci Transl Med. 2023;15(722):eadh9562.
111. McGrail DJ, Pilie PG, Rashid NU, Voorwerk L, Slagter M, Kok M, et al. High tumor mutation burden fails to predict immune checkpoint blockade response across all cancer types. Ann Oncol. 2021;32(5):661–72.
112. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord JP, et al. Efficacy of Pembrolizumab in patients with Noncolorectal high microsatellite Instability/Mismatch repair-deficient Cancer: results from the phase II KEYNOTE-158 study. J Clin Oncol. 2020;38(1):1–10.
113. Darragh LB, Karam SD. Amateur antigen-presenting cells in the tumor microenvironment. Mol Carcinog. 2022;61(2):153–64.
114. Wculek SK, Cueto FJ, Mujal AM, Melero I, Krummel MF, Sancho D. Dendritic cells in cancer immunology and immunotherapy. Nat Rev Immunol. 2020;20(1):7–24.
115. Roberts EW, Broz ML, Binnewies M, Headley MB, Nelson AE, Wolf DM, et al. Critical role for CD103(+)/CD141(+) dendritic cells bearing CCR7 for Tumor Antigen Trafficking and priming of T cell immunity in Melanoma. Cancer Cell. 2016;30(2):324–36.
116. Noman MZ, Parpal S, Van Moer K, Xiao M, Yu Y, Viklund J, et al. Inhibition of Vps34 reprograms cold into hot inflamed tumors and improves anti-PD-1/PD-L1 immunotherapy. Sci Adv. 2020;6(18):eaax7881.
117. Aaes TL, Kaczmarek A, Delvaeye T, De Craene B, De Koker S, Heyndrickx L, et al. Vaccination with Necroptotic Cancer cells induces efficient anti-tumor immunity. Cell Rep. 2016;15(2):274–87.

118. Bell E. CLEC9A: linking necrosis and immunity. Nat Rev Immunol. 2009;9(4):223.
119. Hayashi K, Nikolos F, Chan KS. Inhibitory DAMPs in immunogenic cell death and its clinical implications. Cell Stress. 2021;5(4):52–4.
120. Nikolos F, Hayashi K, Hoi XP, Alonzo ME, Mo Q, Kasabyan A, et al. Cell death-induced immunogenicity enhances chemoimmunotherapeutic response by converting immune-excluded into T-cell inflamed bladder tumors. Nat Commun. 2022;13(1):1487.
121. Wang D, DuBois RN. Immunosuppression associated with chronic inflammation in the tumor microenvironment. Carcinogenesis. 2015;36(10):1085–93.
122. Maier B, Leader AM, Chen ST, Tung N, Chang C, LeBerichel J, et al. A conserved dendritic-cell regulatory program limits antitumour immunity. Nature. 2020;580(7802):257–62.
123. Thumkeo D, Punyawatthananukool S, Prasongtanakij S, Matsuura R, Arima K, Nie H, et al. PGE(2)-EP2/EP4 signaling elicits immunosuppression by driving the mregDC-Treg axis in inflammatory tumor microenvironment. Cell Rep. 2022;39(10):110914.
124. Papaspyridonos M, Matei I, Huang Y, do Rosario Andre M, Brazier-Mitouart H, Waite JC, et al. Id1 suppresses anti-tumour immune responses and promotes tumour progression by impairing myeloid cell maturation. Nat Commun. 2015;6:6840.
125. Yang AS, Lattime EC. Tumor-induced interleukin 10 suppresses the ability of splenic dendritic cells to stimulate CD4 and CD8 T-cell responses. Cancer Res. 2003;63(9):2150–7.
126. Pittet MJ, Di Pilato M, Garris C, Mempel TR. Dendritic cells as shepherds of T cell immunity in cancer. Immunity. 2023;56(10):2218–30.
127. Barry KC, Hsu J, Broz ML, Cueto FJ, Binnewies M, Combes AJ, et al. A natural killer-dendritic cell axis defines checkpoint therapy-responsive tumor microenvironments. Nat Med. 2018;24(8):1178–91.
128. Bottcher JP, Bonavita E, Chakravarty P, Blees H, Cabeza-Cabrerizo M, Sammicheli S, et al. NK Cells Stimulate Recruitment of cDC1 into the Tumor Microenvironment promoting Cancer Immune Control. Cell. 2018;172(5):1022–e3714.
129. Dangaj D, Bruand M, Grimm AJ, Ronet C, Barras D, Duttagupta PA, et al. Cooperation between constitutive and Inducible Chemokines Enables T Cell Engraftment and Immune Attack in Solid tumors. Cancer Cell. 2019;35(6):885–e90010.
130. Mikucki ME, Fisher DT, Matsuzaki J, Skitzki JJ, Gaulin NB, Muhitch JB, et al. Non-redundant requirement for CXCR3 signalling during tumoricidal T-cell trafficking across tumour vascular checkpoints. Nat Commun. 2015;6:7458.
131. Spranger S, Dai D, Horton B, Gajewski TF. Tumor-residing Batf3 dendritic cells are required for effector T cell trafficking and adoptive T cell therapy. Cancer Cell. 2017;31(5):711–23. e4.
132. Ma Y, Liu Y, Zhi Y, Wang H, Yang M, Niu J, et al. Delivery of CXCL9/10/11 plasmid DNAs promotes the tumor-infiltration of T cells and synergizes with PD1 antibody for treating lung cancer. Cancer Nano. 2022;13(1):10.
133. Terhorst D, Fossum E, Baranska A, Tamoutounour S, Malosse C, Garbani M, et al. Laser-assisted intradermal delivery of adjuvant-free vaccines targeting XCR1 + dendritic cells induces potent antitumoral responses. J Immunol. 2015;194(12):5895–902.
134. Zheng W, Skowron KB, Namm JP, Burnette B, Fernandez C, Arina A, et al. Combination of radiotherapy and vaccination overcomes checkpoint blockade resistance. Oncotarget. 2016;7(28):43039–51.
135. Jhunjhunwala S, Hammer C, Delamarre L. Antigen presentation in cancer: insights into tumour immunogenicity and immune evasion. Nat Rev Cancer. 2021;21(5):298–312.
136. Zhou Y-F, Xiao Y, Jin X, Di G-H, Jiang Y-Z, Shao Z-M. Integrated analysis reveals prognostic value of HLA-I LOH in triple-negative breast cancer. J Immunother Cancer. 2021;9(10):e003371.
137. Yamamoto K, Venida A, Yano J, Biancur DE, Kakiuchi M, Gupta S, et al. Autophagy promotes immune evasion of pancreatic cancer by degrading MHC-I. Nature. 2020;581(7806):100–5.
138. Cheung PF, Yang J, Fang R, Borgers A, Krengel K, Stoffel A, et al. Progranulin mediates immune evasion of pancreatic ductal adenocarcinoma through regulation of MHCI expression. Nat Commun. 2022;13(1):156.
139. Belderbos RA, Baas P, Berardi R, Cornelissen R, Fennell DA, van Meerbeeck JP, et al. A multicenter, randomized, phase II/III study of dendritic cells loaded with allogeneic tumor cell lysate (MesoPher) in subjects with mesothelioma as maintenance therapy after chemotherapy: DENdritic cell immunotherapy for Mesothelioma (DENIM) trial. Transl Lung Cancer Res. 2019;8(3):280–5.
140. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat Rev Immunol. 2007;7(9):678–89.
141. Blanchard L, Girard JP. High endothelial venules (HEVs) in immunity, inflammation and cancer. Angiogenesis. 2021;24(4):719–53.
142. Park HR, Shiva A, Cummings P, Kim S, Kim S, Lee E, et al. Angiopoietin-2-Dependent spatial vascular destabilization promotes T-cell exclusion and limits Immunotherapy in Melanoma. Cancer Res. 2023;83(12):1968–83.
143. Lv H, Zong Q, Chen C, Lv G, Xiang W, Xing F, et al. TET2-mediated tumor cGAS triggers endothelial STING activation to regulate vasculature remodeling and anti-tumor immunity in liver cancer. Nat Commun. 2024;15(1):6.
144. Falcon BL, Chintharlapalli S, Uhlik MT, Pytowski B. Antagonist antibodies to vascular endothelial growth factor receptor 2 (VEGFR-2) as anti-angiogenic agents. Pharmacol Ther. 2016;164:204–25.
145. Park JA, Espinosa-Cotton M, Guo H-f, Monette S, Cheung N-KV. Targeting tumor vasculature to improve antitumor activity of T cells armed ex vivo with T cell engaging bispecific antibody. J Immunother Cancer. 2023;11(3):e006680.
146. Martinet L, Garrido I, Filleron T, Le Guellec S, Bellard E, Fournie JJ, et al. Human solid tumors contain high endothelial venules: association with T- and B-lymphocyte infiltration and favorable prognosis in breast cancer. Cancer Res. 2011;71(17):5678–87.
147. Asrir A, Tardiveau C, Coudert J, Laffont R, Blanchard L, Bellard E, et al. Tumor-associated high endothelial venules mediate lymphocyte entry into tumors and predict response to PD-1 plus CTLA-4 combination immunotherapy. Cancer Cell. 2022;40(3):318–34. e9.
148. Yang J, Yan J, Liu B. Targeting VEGF/VEGFR to modulate Antitumor Immunity. Front Immunol. 2018;9:978.
149. Allen E, Jabouille A, Rivera LB, Lodewijckx I, Missiaen R, Steri V, et al. Combined antiangiogenic and anti-PD-L1 therapy stimulates tumor immunity through HEV formation. Sci Transl Med. 2017;9(385):eaak9679.
150. Chuckran CA, Liu C, Bruno TC, Workman CJ, Vignali DA. Neuropilin-1: a checkpoint target with unique implications for cancer immunology and immunotherapy. J Immunother Cancer. 2020;8(2):e000967.
151. Bordry N, Broggi MAS, de Jonge K, Schaeuble K, Gannon PO, Foukas PG, et al. Lymphatic vessel density is associated with CD8(+) T cell infiltration and immunosuppressive factors in human melanoma. Oncoimmunology. 2018;7(8):e1462878.
152. Steele MM, Jaiswal A, Delclaux I, Dryg ID, Murugan D, Femel J, et al. T cell egress via lymphatic vessels is tuned by antigen encounter and limits tumor control. Nat Immunol. 2023;24(4):664–75.
153. Steele MM, Lund AW. Afferent Lymphatic Transport and peripheral tissue immunity. J Immunol. 2021;206(2):264–72.
154. Eisenbarth SC. Dendritic cell subsets in T cell programming: location dictates function. Nat Rev Immunol. 2019;19(2):89–103.
155. Feig C, Jones JO, Kraman M, Wells RJ, Deonarine A, Chan DS, et al. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. Proc Natl Acad Sci U S A. 2013;110(50):20212–7.
156. Wang Z, Moresco P, Yan R, Li J, Gao Y, Biasci D, et al. Carcinomas assemble a filamentous CXCL12-keratin-19 coating that suppresses T cell-mediated immune attack. Proc Natl Acad Sci U S A. 2022;119(4):e2119463119.
157. Li Z, Tuong ZK, Dean I, Willis C, Gaspal F, Fiancette R, et al. In vivo labeling reveals continuous trafficking of TCF-1 + T cells between tumor and lymphoid tissue. J Exp Med. 2022;219(6):e20210749.
158. Lane RS, Femel J, Breazeale AP, Loo CP, Thibault G, Kaempf A, et al. IFNgamma-activated dermal lymphatic vessels inhibit cytotoxic T cells in melanoma and inflamed skin. J Exp Med. 2018;215(12):3057–74.
159. Domblides C, Rochefort J, Riffard C, Panouillot M, Lescaille G, Teillaud JL, et al. Tumor-Associated Tertiary lymphoid structures: from Basic and clinical knowledge to therapeutic manipulation. Front Immunol. 2021;12:698604.
160. Wang Q, Zhi Y, Zi M, Mo Y, Wang Y, Liao Q, et al. Spatially resolved Transcriptomics Technology facilitates Cancer Research. Adv Sci (Weinh). 2023;10(30):e2302558.
161. Kasikova L, Rakova J, Hensler M, Lanickova T, Tomankova J, Pasulka J, et al. Tertiary lymphoid structures and B cells determine clinically relevant T cell phenotypes in ovarian cancer. Nat Commun. 2024;15(1):2528.
162. Dieu-Nosjean MC, Goc J, Giraldo NA, Sautes-Fridman C, Fridman WH. Tertiary lymphoid structures in cancer and beyond. Trends Immunol. 2014;35(11):571–80.
163. Lin Q, Tao P, Wang J, Ma L, Jiang Q, Li J, et al. Tumor-associated tertiary lymphoid structure predicts postoperative outcomes in patients with primary gastrointestinal stromal tumors. Oncoimmunology. 2020;9(1):1747339.

164. Dieu-Nosjean MC, Antoine M, Danel C, Heudes D, Wislez M, Poulot V, et al. Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. J Clin Oncol. 2008;26(27):4410–7.
165. Calderaro J, Petitprez F, Becht E, Laurent A, Hirsch TZ, Rousseau B, et al. Intratumoral tertiary lymphoid structures are associated with a low risk of early recurrence of hepatocellular carcinoma. J Hepatol. 2019;70(1):58–65.
166. Cabrita R, Lauss M, Sanna A, Donia M, Skaarup Larsen M, Mitra S, et al. Tertiary lymphoid structures improve immunotherapy and survival in melanoma. Nature. 2020;577(7791):561–5.
167. Wang Q, Sun K, Liu R, Song Y, Lv Y, Bi P, et al. Single-cell transcriptome sequencing of B-cell heterogeneity and tertiary lymphoid structure predicts breast cancer prognosis and neoadjuvant therapy efficacy. Clin Transl Med. 2023;13(8):e1346.
168. Posch F, Silina K, Leibl S, Mundlein A, Moch H, Siebenhuner A, et al. Maturation of tertiary lymphoid structures and recurrence of stage II and III colorectal cancer. Oncoimmunology. 2018;7(2):e1378844.
169. Rakaee M, Kilvaer TK, Jamaly S, Berg T, Paulsen EE, Berglund M, et al. Tertiary lymphoid structure score: a promising approach to refine the TNM staging in resected non-small cell lung cancer. Br J Cancer. 2021;124(10):1680–9.
170. Vanhersecke L, Brunet M, Guegan JP, Rey C, Bougouin A, Cousin S, et al. Mature tertiary lymphoid structures predict immune checkpoint inhibitor efficacy in solid tumors independently of PD-L1 expression. Nat Cancer. 2021;2(8):794–802.
171. Feng X, Tonon L, Li H, Darbo E, Pleasance E, Macagno N, et al. Comprehensive Immune Profiling unveils a subset of Leiomyosarcoma with Hot Tumor Immune Microenvironment. Cancers. 2023;15(14):3705.
172. Ding GY, Ma JQ, Yun JP, Chen X, Ling Y, Zhang S, et al. Distribution and density of tertiary lymphoid structures predict clinical outcome in intrahepatic cholangiocarcinoma. J Hepatol. 2022;76(3):608–18.
173. Wang Q, Shen X, An R, Bai J, Dong J, Cai H, et al. Peritumoral tertiary lymphoid structure and tumor stroma percentage predict the prognosis of patients with non-metastatic colorectal cancer. Front Immunol. 2022;13:962056.
174. Edmonds NL, Gradecki SE, Katyal P, Lynch KT, Stowman AM, Gru AA, et al. Tertiary lymphoid structures in desmoplastic melanoma have increased lymphocyte density, lymphocyte proliferation, and immune cross talk with tumor when compared to non-desmoplastic melanomas. Oncoimmunology. 2023;12(1):2164476.
175. Lauss M, Donia M, Svane IM, Jonsson G. B cells and tertiary lymphoid structures: friends or foes in Cancer Immunotherapy? Clin Cancer Res. 2022;28(9):1751–8.
176. Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, et al. B cells and tertiary lymphoid structures promote immunotherapy response. Nature. 2020;577(7791):549–55.
177. Sautes-Fridman C, Verneau J, Sun CM, Moreira M, Chen TW, Meylan M, et al. Tertiary lymphoid structures and B cells: clinical impact and therapeutic modulation in cancer. Semin Immunol. 2020;48:101406.
178. Wouters MCA, Nelson BH. Prognostic significance of Tumor-infiltrating B cells and plasma cells in Human Cancer. Clin Cancer Res. 2018;24(24):6125–35.
179. Kinker GS, Vitiello GAF, Diniz AB, Cabral-Piccin MP, Pereira PHB, Carvalho MLR, et al. Mature tertiary lymphoid structures are key niches of tumour-specific immune responses in pancreatic ductal adenocarcinomas. Gut. 2023;72(10):1927–41.
180. Meylan M, Petitprez F, Becht E, Bougouin A, Pupier G, Calvez A, et al. Tertiary lymphoid structures generate and propagate anti-tumor antibody-producing plasma cells in renal cell cancer. Immunity. 2022;55(3):527–41. e5.
181. Lutz ER, Wu AA, Bigelow E, Sharma R, Mo G, Soares K, et al. Immunotherapy converts nonimmunogenic pancreatic tumors into immunogenic foci of immune regulation. Cancer Immunol Res. 2014;2(7):616–31.
182. Maldonado L, Teague JE, Morrow MP, Jotova I, Wu TC, Wang C, et al. Intramuscular therapeutic vaccination targeting HPV16 induces T cell responses that localize in mucosal lesions. Sci Transl Med. 2014;6(221):221ra13.
183. Fridman WH, Petitprez F, Meylan M, Chen TW, Sun CM, Roumenina LT, et al. B cells and cancer: to B or not to B? J Exp Med. 2021;218(1):e20200851.
184. Torcellan T, Hampton HR, Bailey J, Tomura M, Brink R, Chtanova T. In vivo photolabeling of tumor-infiltrating cells reveals highly regulated egress of T-cell subsets from tumors. Proc Natl Acad Sci U S A. 2017;114(22):5677–82.
185. Hunter MC, Teijeira A, Halin C. T cell trafficking through lymphatic vessels. Front Immunol. 2016;7:613.
186. Nirmal AJ, Maliga Z, Vallius T, Quattrochi B, Chen AA, Jacobson CA, et al. The spatial Landscape of Progression and Immunoediting in primary melanoma at single-cell resolution. Cancer Discov. 2022;12(6):1518–41.
187. Tie Y, Tang F, Wei YQ, Wei XW. Immunosuppressive cells in cancer: mechanisms and potential therapeutic targets. J Hematol Oncol. 2022;15(1):61.
188. Veglia F, Sanseviero E, Gabrilovich DI. Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. Nat Rev Immunol. 2021;21(8):485–98.
189. Wu Y, Yi M, Niu M, Mei Q, Wu K. Myeloid-derived suppressor cells: an emerging target for anticancer immunotherapy. Mol Cancer. 2022;21(1):184.
190. Safarzadeh E, Orangi M, Mohammadi H, Babaie F, Baradaran B. Myeloid-derived suppressor cells: important contributors to tumor progression and metastasis. J Cell Physiol. 2018;233(4):3024–36.
191. Hillmer EJ, Zhang H, Li HS, Watowich SS. STAT3 signaling in immunity. Cytokine Growth Factor Rev. 2016;31:1–15.
192. Xu W, Li S, Li M, Yang X, Xie S, Lin L, et al. Targeted elimination of myeloid-derived suppressor cells via regulation of the STAT pathway alleviates tumor immunosuppression in neuroblastoma. Immunol Lett. 2021;240:31–40.
193. Lu J, Luo Y, Rao D, Wang T, Lei Z, Chen X, et al. Myeloid-derived suppressor cells in cancer: therapeutic targets to overcome tumor immune evasion. Exp Hematol Oncol. 2024;13(1):39.
194. de Coana YP, Wolodarski M, Poschke I, Yoshimoto Y, Yang Y, Nystrom M, et al. Ipilimumab treatment decreases monocytic MDSCs and increases CD8 effector memory T cells in long-term survivors with advanced melanoma. Oncotarget. 2017;8(13):21539–53.
195. Mortezaee K. Myeloid-derived suppressor cells in cancer immunotherapy-clinical perspectives. Life Sci. 2021;277:119627.
196. Li J, Byrne KT, Yan F, Yamazoe T, Chen Z, Baslan T, et al. Tumor Cell-intrinsic factors underlie heterogeneity of Immune Cell infiltration and response to Immunotherapy. Immunity. 2018;49(1):178–93. e7.
197. Bruni S, Mercogliano MF, Mauro FL, Cordo Russo RI, Schillaci R. Cancer immune exclusion: breaking the barricade for a successful immunotherapy. Front Oncol. 2023;13:1135456.
198. Molon B, Ugel S, Del Pozzo F, Soldani C, Zilio S, Avella D, et al. Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells. J Exp Med. 2011;208(10):1949–62.
199. Li K, Shi H, Zhang B, Ou X, Ma Q, Chen Y, et al. Myeloid-derived suppressor cells as immunosuppressive regulators and therapeutic targets in cancer. Signal Transduct Target Ther. 2021;6(1):362.
200. Yamada M, Yanaba K, Hasegawa M, Matsushita Y, Horikawa M, Komura K, et al. Regulation of local and metastatic host-mediated anti-tumour mechanisms by L-selectin and intercellular adhesion molecule-1. Clin Exp Immunol. 2006;143(2):216–27.
201. Noman MZ, Desantis G, Janji B, Hasmim M, Karray S, Dessen P, et al. PD-L1 is a novel direct target of HIF-1alpha, and its blockade under hypoxia enhanced MDSC-mediated T cell activation. J Exp Med. 2014;211(5):781–90.
202. Zhang B, Wang Z, Wu L, Zhang M, Li W, Ding J, et al. Circulating and tumor-infiltrating myeloid-derived suppressor cells in patients with colorectal carcinoma. PLoS ONE. 2013;8(2):e57114.
203. Iwata T, Kondo Y, Kimura O, Morosawa T, Fujisaka Y, Umetsu T, et al. PD-L1(+) MDSCs are increased in HCC patients and induced by soluble factor in the tumor microenvironment. Sci Rep. 2016;6:39296.
204. Yamamoto S, Kato M, Takeyama Y, Azuma Y, Yukimatsu N, Hirayama Y, et al. Irradiation plus myeloid-derived suppressor cell-targeted therapy for overcoming treatment resistance in immunologically cold urothelial carcinoma. Br J Cancer. 2023;128(12):2197–205.
205. Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. Nat Rev Immunol. 2018;18(2):134–47.
206. Teijeira A, Garasa S, Gato M, Alfaro C, Migueliz I, Cirella A, et al. CXCR1 and CXCR2 chemokine receptor agonists produced by tumors induce Neutrophil Extracellular traps that interfere with Immune cytotoxicity. Immunity. 2020;52(5):856–71. e8.
207. Taifour T, Attalla SS, Zuo D, Gu Y, Sanguin-Gendreau V, Proud H, et al. The tumor-derived cytokine Chi3l1 induces neutrophil extracellular traps that promote T cell exclusion in triple-negative breast cancer. Immunity. 2023;56(12):2755–e728.
208. Zhang Y, Chandra V, Riquelme Sanchez E, Dutta P, Quesada PR, Rakoski A, et al. Interleukin-17-induced neutrophil extracellular traps mediate resistance to checkpoint blockade in pancreatic cancer. J Exp Med. 2020;217(12):e20190354.
209. Kaltenmeier C, Yazdani HO, Morder K, Geller DA, Simmons RL, Tohme S. Neutrophil Extracellular traps promote T cell exhaustion in the Tumor Microenvironment. Front Immunol. 2021;12:785222.
210. Shinde-Jadhav S, Mansure JJ, Rayes RF, Marcq G, Ayoub M, Skowronski R, et al. Role of neutrophil extracellular traps in radiation resistance of invasive bladder cancer. Nat Commun. 2021;12(1):2776.

211. Liu Y, Liang S, Jiang D, Gao T, Fang Y, Fu S, et al. Manipulation of TAMs functions to facilitate the immune therapy effects of immune checkpoint antibodies. J Control Release. 2021;336:621–34.
212. Wang L, He T, Liu J, Tai J, Wang B, Chen Z, et al. Pan-cancer analysis reveals tumor-associated macrophage communication in the tumor microenvironment. Exp Hematol Oncol. 2021;10(1):31.
213. Zhu S, Yi M, Wu Y, Dong B, Wu K. Roles of tumor-associated macrophages in tumor progression: implications on therapeutic strategies. Exp Hematol Oncol. 2021;10(1):60.
214. Kersten K, Hu KH, Combes AJ, Samad B, Harwin T, Ray A, et al. Spatiotemporal co-dependency between macrophages and exhausted CD8(+) T cells in cancer. Cancer Cell. 2022;40(6):624–38. e9.
215. Boutilier AJ, Elsawa SF. Macrophage polarization States in the Tumor Microenvironment. Int J Mol Sci. 2021;22(13):6995.
216. Peranzoni E, Lemoine J, Vimeux L, Feuillet V, Barrin S, Kantari-Mimoun C, et al. Macrophages impede CD8 T cells from reaching tumor cells and limit the efficacy of anti-PD-1 treatment. Proc Natl Acad Sci U S A. 2018;115(17):E4041–50.
217. Liang Y, Tan Y, Guan B, Guo B, Xia M, Li J, et al. Single-cell atlases link macrophages and CD8(+) T-cell subpopulations to disease progression and immunotherapy response in urothelial carcinoma. Theranostics. 2022;12(18):7745–59.
218. Antoranz A, Van Herck Y, Bolognesi MM, Lynch SM, Rahman A, Gallagher WM, et al. Mapping the Immune Landscape in Metastatic Melanoma reveals localized cell-cell interactions that predict Immunotherapy Response. Cancer Res. 2022;82(18):3275–90.
219. Su S, Liao J, Liu J, Huang D, He C, Chen F, et al. Blocking the recruitment of naive CD4(+) T cells reverses immunosuppression in breast cancer. Cell Res. 2017;27(4):461–82.
220. Kloosterman DJ, Akkari L. Macrophages at the interface of the co-evolving cancer ecosystem. Cell. 2023;186(8):1627–51.
221. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med. 2004;10(9):942–9.
222. Liu J, Zhang N, Li Q, Zhang W, Ke F, Leng Q, et al. Tumor-associated macrophages recruit CCR6 + regulatory T cells and promote the development of colorectal cancer via enhancing CCL20 production in mice. PLoS ONE. 2011;6(4):e19495.
223. Shang S, Yang C, Chen F, Xiang RS, Zhang H, Dai SY, et al. ID1 expressing macrophages support cancer cell stemness and limit CD8(+) T cell infiltration in colorectal cancer. Nat Commun. 2023;14(1):7661.
224. Ning J, Ye Y, Shen H, Zhang R, Li H, Song T, et al. Macrophage-coated tumor cluster aggravates hepatoma invasion and immunotherapy resistance via generating local immune deprivation. Cell Rep Med. 2024;5(5):101505.
225. Chen Y, Song Y, Du W, Gong L, Chang H, Zou Z. Tumor-associated macrophages: an accomplice in solid tumor progression. J Biomed Sci. 2019;26(1):78.
226. Klichinsky M, Ruella M, Shestova O, Lu XM, Best A, Zeeman M, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. Nat Biotechnol. 2020;38(8):947–53.
227. Zhang L, Tian L, Dai X, Yu H, Wang J, Lei A, et al. Pluripotent stem cell-derived CAR-macrophage cells with antigen-dependent anti-cancer cell functions. J Hematol Oncol. 2020;13(1):153.
228. Wang Y, Johnson KCC, Gatti-Mays ME, Li Z. Emerging strategies in targeting tumor-resident myeloid cells for cancer immunotherapy. J Hematol Oncol. 2022;15(1):118.
229. Netea-Maier RT, Smit JWA, Netea MG. Metabolic changes in tumor cells and tumor-associated macrophages: a mutual relationship. Cancer Lett. 2018;413:102–9.
230. Holmgaard RB, Zamarin D, Li Y, Gasmi B, Munn DH, Allison JP, et al. Tumor-expressed IDO recruits and activates MDSCs in a Treg-Dependent Manner. Cell Rep. 2015;13(2):412–24.
231. Holmgaard RB, Zamarin D, Lesokhin A, Merghoub T, Wolchok JD. Targeting myeloid-derived suppressor cells with colony stimulating factor-1 receptor blockade can reverse immune resistance to immunotherapy in indoleamine 2,3-dioxygenase-expressing tumors. EBioMedicine. 2016;6:50–8.
232. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol. 2012;12(4):253–68.
233. Smith C, Chang MY, Parker KH, Beury DW, DuHadaway JB, Flick HE, et al. IDO is a nodal pathogenic driver of lung cancer and metastasis development. Cancer Discov. 2012;2(8):722–35.
234. Moon YW, Hajjar J, Hwu P, Naing A. Targeting the indoleamine 2,3-dioxygenase pathway in cancer. J Immunother Cancer. 2015;3:51.
235. Spranger S, Spaapen RM, Zha Y, Williams J, Meng Y, Ha TT, et al. Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. Sci Transl Med. 2013;5(200):200ra116.
236. Fu R, Zhang YW, Li HM, Lv WC, Zhao L, Guo QL, et al. LW106, a novel indoleamine 2,3-dioxygenase 1 inhibitor, suppresses tumour progression by limiting stroma-immune crosstalk and cancer stem cell enrichment in tumour micro-environment. Br J Pharmacol. 2018;175(14):3034–49.
237. Jones D, Wang Z, Chen IX, Zhang S, Banerji R, Lei PJ, et al. Solid stress impairs lymphocyte infiltration into lymph-node metastases. Nat Biomed Eng. 2021;5(12):1426–36.
238. Tauriello DVF, Palomo-Ponce S, Stork D, Berenguer-Llergo A, Badia-Ramentol J, Iglesias M, et al. TGFbeta drives immune evasion in genetically reconstituted colon cancer metastasis. Nature. 2018;554(7693):538–43.
239. Thomas D, Radhakrishnan P. Tumor-stromal crosstalk in pancreatic cancer and tissue fibrosis. Mol Cancer. 2019;18(1):14.
240. Melssen MM, Sheybani ND, Leick KM, Slingluff CL. Jr. Barriers to immune cell infiltration in tumors. J Immunother Cancer. 2023;11(4):e006401.
241. Peng DH, Rodriguez BL, Diao L, Chen L, Wang J, Byers LA, et al. Collagen promotes anti-PD-1/PD-L1 resistance in cancer through LAIR1-dependent CD8(+) T cell exhaustion. Nat Commun. 2020;11(1):4520.
242. Lynch CC. Matrix metalloproteinases as master regulators of the vicious cycle of bone metastasis. Bone. 2011;48(1):44–53.
243. Kaur A, Ecker BL, Douglass SM, Kugel CH 3rd, Webster MR, Almeida FV, et al. Remodeling of the Collagen Matrix in aging skin promotes Melanoma Metastasis and affects Immune Cell Motility. Cancer Discov. 2019;9(1):64–81.
244. Nissen NI, Karsdal M, Willumsen N. Collagens and Cancer associated fibroblasts in the reactive stroma and its relation to Cancer biology. J Exp Clin Cancer Res. 2019;38(1):115.
245. Fields GB. The rebirth of Matrix Metalloproteinase inhibitors: moving beyond the Dogma. Cells. 2019;8(9):984.
246. Monteran L, Erez N. The Dark side of fibroblasts: Cancer-Associated fibroblasts as mediators of Immunosuppression in the Tumor Microenvironment. Front Immunol. 2019;10:1835.
247. Xiao Z, Todd L, Huang L, Noguera-Ortega E, Lu Z, Huang L, et al. Desmoplastic stroma restricts T cell extravasation and mediates immune exclusion and immunosuppression in solid tumors. Nat Commun. 2023;14(1):5110.
248. Grout JA, Sirven P, Leader AM, Maskey S, Hector E, Puisieux I, et al. Spatial positioning and Matrix Programs of Cancer-Associated fibroblasts promote T-cell exclusion in human lung tumors. Cancer Discov. 2022;12(11):2606–25.
249. You T, Tang H, Wu W, Gao J, Li X, Li N, et al. POSTN Secretion by Extracellular Matrix Cancer-Associated fibroblasts (eCAFs) correlates with poor ICB response via Macrophage Chemotaxis activation of akt signaling pathway in gastric Cancer. Aging Dis. 2023;14(6):2177–92.
250. Nagaraj S, Gupta K, Pisarev V, Kinarsky L, Sherman S, Kang L, et al. Altered recognition of antigen is a mechanism of CD8 + T cell tolerance in cancer. Nat Med. 2007;13(7):828–35.
251. Neuzillet C, Nicolle R, Raffenne J, Tijeras-Raballand A, Brunel A, Astorgues-Xerri L, et al. Periostin- and podoplanin-positive cancer-associated fibroblast subtypes cooperate to shape the inflamed tumor microenvironment in aggressive pancreatic adenocarcinoma. J Pathol. 2022;258(4):408–25.
252. Yu T, Di G. Role of tumor microenvironment in triple-negative breast cancer and its prognostic significance. Chin J Cancer Res. 2017;29(3):237–52.
253. Malanchi I, Santamaria-Martinez A, Susanto E, Peng H, Lehr HA, Delaloye JF, et al. Interactions between cancer stem cells and their niche govern metastatic colonization. Nature. 2011;481(7379):85–9.
254. Hanahan D. Hallmarks of Cancer: New dimensions. Cancer Discov. 2022;12(1):31–46.
255. Fane M, Weeraratna AT. How the ageing microenvironment influences tumour progression. Nat Rev Cancer. 2020;20(2):89–106.
256. Faget DV, Ren Q, Stewart SA. Unmasking senescence: context-dependent effects of SASP in cancer. Nat Rev Cancer. 2019;19(8):439–53.
257. Gabai Y, Assouline B, Ben-Porath I. Senescent stromal cells: roles in the tumor microenvironment. Trends Cancer. 2023;9(1):28–41.
258. Zhao B, Wu B, Feng N, Zhang X, Zhang X, Wei Y, et al. Aging microenvironment and antitumor immunity for geriatric oncology: the landscape and future implications. J Hematol Oncol. 2023;16(1):28.
259. Coppe JP, Desprez PY, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu Rev Pathol. 2010;5:99–118.

260. Zhou P, Liu Z, Hu H, Lu Y, Xiao J, Wang Y, et al. Comprehensive Analysis of Senescence Characteristics defines a Novel Prognostic signature to Guide Personalized Treatment for Clear Cell Renal Cell Carcinoma. Front Immunol. 2022;13:901671.
261. Tauriello DVF, Sancho E, Batlle E. Overcoming TGFβ-mediated immune evasion in cancer. Nat Rev Cancer. 2022;22(1):25–44.
262. Mao X, Xu J, Wang W, Liang C, Hua J, Liu J, et al. Crosstalk between cancer-associated fibroblasts and immune cells in the tumor microenvironment: new findings and future perspectives. Mol Cancer. 2021;20(1):131.
263. Zhang J, Shi Z, Xu X, Yu Z, Mi J. The influence of microenvironment on tumor immunotherapy. FEBS J. 2019;286(21):4160–75.
264. Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature. 2001;410(6824):50–6.
265. Farhangnia P, Khorramdelazad H, Nickho H, Delbandi AA. Current and future immunotherapeutic approaches in pancreatic cancer treatment. J Hematol Oncol. 2024;17(1):40.
266. Ford K, Hanley CJ, Mellone M, Szyndralewiez C, Heitz F, Wiesel P, et al. NOX4 inhibition potentiates immunotherapy by overcoming Cancer-Associated fibroblast-mediated CD8 T-cell exclusion from tumors. Cancer Res. 2020;80(9):1846–60.
267. Grauel AL, Nguyen B, Ruddy D, Laszewski T, Schwartz S, Chang J, et al. TGFbeta-blockade uncovers stromal plasticity in tumors by revealing the existence of a subset of interferon-licensed fibroblasts. Nat Commun. 2020;11(1):6315.
268. Witherel CE, Sao K, Brisson BK, Han B, Volk SW, Petrie RJ, et al. Regulation of extracellular matrix assembly and structure by hybrid M1/M2 macrophages. Biomaterials. 2021;269:120667.
269. Liu Y, Xun Z, Ma K, Liang S, Li X, Zhou S, et al. Identification of a tumour immune barrier in the HCC microenvironment that determines the efficacy of immunotherapy. J Hepatol. 2023;78(4):770–82.
270. Qi J, Sun H, Zhang Y, Wang Z, Xun Z, Li Z, et al. Single-cell and spatial analysis reveal interaction of FAP(+) fibroblasts and SPP1(+) macrophages in colorectal cancer. Nat Commun. 2022;13(1):1742.
271. Sun X, He X, Zhang Y, Hosaka K, Andersson P, Wu J, et al. Inflammatory cell-derived CXCL3 promotes pancreatic cancer metastasis through a novel myofibroblast-hijacked cancer escape mechanism. Gut. 2022;71(1):129–47.
272. Roberts EW, Deonarine A, Jones JO, Denton AE, Feig C, Lyons SK, et al. Depletion of stromal cells expressing fibroblast activation protein-alpha from skeletal muscle and bone marrow results in cachexia and anemia. J Exp Med. 2013;210(6):1137–51.
273. Cremasco V, Astarita JL, Grauel AL, Keerthivasan S, MacIsaac K, Woodruff MC, et al. FAP delineates heterogeneous and functionally divergent stromal cells in Immune-excluded breast tumors. Cancer Immunol Res. 2018;6(12):1472–85.
274. Sherman MH, Yu RT, Engle DD, Ding N, Atkins AR, Tiriac H, et al. Vitamin D receptor-mediated stromal reprogramming suppresses pancreatitis and enhances pancreatic cancer therapy. Cell. 2014;159(1):80–93.
275. Elia I, Haigis MC. Metabolites and the tumour microenvironment: from cellular mechanisms to systemic metabolism. Nat Metab. 2021;3(1):21–32.
276. Vander Heiden MG, DeBerardinis RJ. Understanding the intersections between Metabolism and Cancer Biology. Cell. 2017;168(4):657–69.
277. Zhang C, Chen S, Ma X, Yang Q, Su F, Shu X, et al. Upregulation of STC2 in colorectal cancer and its clinicopathological significance. Onco Targets Ther. 2019;12:1249–58.
278. Hartmann FJ, Mrdjen D, McCaffrey E, Glass DR, Greenwald NF, Bharadwaj A, et al. Single-cell metabolic profiling of human cytotoxic T cells. Nat Biotechnol. 2021;39(2):186–97.
279. Watson MJ, Vignali PDA, Mullett SJ, Overacre-Delgoffe AE, Peralta RM, Grebinoski S, et al. Metabolic support of tumour-infiltrating regulatory T cells by lactic acid. Nature. 2021;591(7851):645–51.
280. Ma L, Craig AJ, Heinrich S. Hypoxia is a key regulator in liver cancer progression. J Hepatol. 2021;75(3):736–7.
281. Milotti E, Fredrich T, Chignola R, Rieger H. Oxygen in the Tumor Microenvironment: Mathematical and Numerical modeling. Adv Exp Med Biol. 2020;1259:53–76.
282. Sugiura A, Rathmell JC. Metabolic barriers to T cell function in tumors. J Immunol. 2018;200(2):400–7.
283. Jayaprakash P, Ai M, Liu A, Budhani P, Bartkowiak T, Sheng J, et al. Targeted hypoxia reduction restores T cell infiltration and sensitizes prostate cancer to immunotherapy. J Clin Invest. 2018;128(11):5137–49.
284. Hatfield SM, Kjaergaard J, Lukashev D, Schreiber TH, Belikoff B, Abbott R, et al. Immunological mechanisms of the antitumor effects of supplemental oxygenation. Sci Transl Med. 2015;7(277):277ra30.
285. Craig SG, Humphries MP, Alderdice M, Bingham V, Richman SD, Loughrey MB, et al. Immune status is prognostic for poor survival in colorectal cancer patients and is associated with tumour hypoxia. Br J Cancer. 2020;123(8):1280–8.
286. Li M, Zhou B, Zheng C. An integrated bioinformatic analysis of bulk and single-cell sequencing clarifies immune microenvironment and metabolic profiles of lung adenocarcinoma to predict immunotherapy efficacy. Front Cell Dev Biol. 2023;11:1163314.
287. Sattiraju A, Kang S, Giotti B, Chen Z, Marallano VJ, Brusco C, et al. Hypoxic niches attract and sequester tumor-associated macrophages and cytotoxic T cells and reprogram them for immunosuppression. Immunity. 2023;56(8):1825–43. e6.
288. Sethumadhavan S, Silva M, Philbrook P, Nguyen T, Hatfield SM, Ohta A, et al. Hypoxia and hypoxia-inducible factor (HIF) downregulate antigen-presenting MHC class I molecules limiting tumor cell recognition by T cells. PLoS ONE. 2017;12(11):e0187314.
289. Murthy A, Gerber SA, Koch CJ, Lord EM. Intratumoral Hypoxia reduces IFN-gamma-mediated immunity and MHC class I induction in a preclinical Tumor Model. Immunohorizons. 2019;3(4):149–60.
290. Yan Y, Huang L, Liu Y, Yi M, Chu Q, Jiao D, et al. Metabolic profiles of regulatory T cells and their adaptations to the tumor microenvironment: implications for antitumor immunity. J Hematol Oncol. 2022;15(1):104.
291. Shi L, Feng M, Du S, Wei X, Song H, Yixin X, et al. Adenosine Generated by Regulatory T Cells induces CD8(+) T cell exhaustion in gastric Cancer through A2aR pathway. Biomed Res Int. 2019;2019:4093214.
292. Scharping NE, Rivadeneira DB, Menk AV, Vignali PDA, Ford BR, Rittenhouse NL, et al. Mitochondrial stress induced by continuous stimulation under hypoxia rapidly drives T cell exhaustion. Nat Immunol. 2021;22(2):205–15.
293. Yuan Z, Li Y, Zhang S, Wang X, Dou H, Yu X, et al. Extracellular matrix remodeling in tumor progression and immune escape: from mechanisms to treatments. Mol Cancer. 2023;22(1):48.
294. Shi X, Yang J, Deng S, Xu H, Wu D, Zeng Q, et al. TGF-beta signaling in the tumor metabolic microenvironment and targeted therapies. J Hematol Oncol. 2022;15(1):135.
295. Bai S, Chen L, Yan Y, Li R, Zhou Y, Wang X, et al. Exploration of different hypoxia patterns and construction of a hypoxia-related gene Prognostic Index in Colorectal Cancer. Front Immunol. 2022;13:853352.
296. Andrejeva G, Rathmell JC. Similarities and distinctions of Cancer and Immune metabolism in inflammation and tumors. Cell Metab. 2017;26(1):49–70.
297. Zhong X, He X, Wang Y, Hu Z, Huang H, Zhao S, et al. Warburg effect in colorectal cancer: the emerging roles in tumor microenvironment and therapeutic implications. J Hematol Oncol. 2022;15(1):160.
298. Ganapathy-Kanniappan S, Geschwind JF. Tumor glycolysis as a target for cancer therapy: progress and prospects. Mol Cancer. 2013;12:152.
299. Gupta R, Liu AY, Glazer PM, Wajapeyee N. LKB1 preserves genome integrity by stimulating BRCA1 expression. Nucleic Acids Res. 2015;43(1):259–71.
300. Faubert B, Vincent EE, Griss T, Samborska B, Izreig S, Svensson RU, et al. Loss of the tumor suppressor LKB1 promotes metabolic reprogramming of cancer cells via HIF-1alpha. Proc Natl Acad Sci U S A. 2014;111(7):2554–9.
301. Qian Y, Galan-Cobo A, Guijarro I, Dang M, Molkentine D, Poteete A, et al. MCT4-dependent lactate secretion suppresses antitumor immunity in LKB1-deficient lung adenocarcinoma. Cancer Cell. 2023;41(7):1363–80. e7.
302. Ottensmeier CH, Perry KL, Harden EL, Stasakova J, Jenei V, Fleming J, et al. Upregulated glucose metabolism correlates inversely with CD8 + T-cell infiltration and survival in squamous cell carcinoma. Cancer Res. 2016;76(14):4136–48.
303. Siska PJ, Beckermann KE, Mason FM, Andrejeva G, Greenplate AR, Sendor AB, et al. Mitochondrial dysregulation and glycolytic insufficiency functionally impair CD8 T cells infiltrating human renal cell carcinoma. JCI Insight. 2017;2(12):e93411.
304. Chang CH, Curtis JD, Maggi LB Jr., Faubert B, Villarino AV, O'Sullivan D, et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. Cell. 2013;153(6):1239–51.
305. Cao J, Liao S, Zeng F, Liao Q, Luo G, Zhou Y. Effects of altered glycolysis levels on CD8(+) T cell activation and function. Cell Death Dis. 2023;14(7):407.
306. Pilon-Thomas S, Kodumudi KN, El-Kenawi AE, Russell S, Weber AM, Luddy K, et al. Neutralization of Tumor Acidity improves antitumor responses to Immunotherapy. Cancer Res. 2016;76(6):1381–90.

307. Kumagai S, Koyama S, Itahashi K, Tanegashima T, Lin YT, Togashi Y, et al. Lactic acid promotes PD-1 expression in regulatory T cells in highly glycolytic tumor microenvironments. Cancer Cell. 2022;40(2):201–18. e9.
308. Kao KC, Vilbois S, Tsai CH, Ho PC. Metabolic communication in the tumour-immune microenvironment. Nat Cell Biol. 2022;24(11):1574–83.
309. Gemta LF, Siska PJ, Nelson ME, Gao X, Liu X, Locasale JW, et al. Impaired enolase 1 glycolytic activity restrains effector functions of tumor-infiltrating CD8(+) T cells. Sci Immunol. 2019;4(31):eaap9520.
310. Chen T, Xu Z-G, Luo J, Manne RK, Wang Z, Hsu C-C, et al. NSUN2 is a glucose sensor suppressing cGAS/STING to maintain tumorigenesis and immunotherapy resistance. Cell Metab. 2023;35(10):1782–e988.
311. Reinfeld BI, Madden MZ, Wolf MM, Chytil A, Bader JE, Patterson AR, et al. Cell-programmed nutrient partitioning in the tumour microenvironment. Nature. 2021;593(7858):282–8.
312. Dey P, Kimmelman AC, DePinho RA. Metabolic codependencies in the Tumor Microenvironment. Cancer Discov. 2021;11(5):1067–81.
313. Yang L, Achreja A, Yeung TL, Mangala LS, Jiang D, Han C, et al. Targeting stromal glutamine synthetase in Tumors disrupts Tumor Microenvironment-Regulated Cancer Cell Growth. Cell Metab. 2016;24(5):685–700.
314. Wang B, Pei J, Xu S, Liu J, Yu J. A glutamine tug-of-war between cancer and immune cells: recent advances in unraveling the ongoing battle. J Exp Clin Cancer Res. 2024;43(1):74.
315. Edwards DN, Ngwa VM, Raybuck AL, Wang S, Hwang Y, Kim LC, et al. Selective glutamine metabolism inhibition in tumor cells improves antitumor T lymphocyte activity in triple-negative breast cancer. J Clin Invest. 2021;131(4):e140100.
316. Wang Q, Hardie RA, Hoy AJ, van Geldermalsen M, Gao D, Fazli L, et al. Targeting ASCT2-mediated glutamine uptake blocks prostate cancer growth and tumour development. J Pathol. 2015;236(3):278–89.
317. Altman BJ, Stine ZE, Dang CV. From Krebs to clinic: glutamine metabolism to cancer therapy. Nat Rev Cancer. 2016;16(11):749.
318. Xiang L, Mou J, Shao B, Wei Y, Liang H, Takano N, et al. Glutaminase 1 expression in colorectal cancer cells is induced by hypoxia and required for tumor growth, invasion, and metastatic colonization. Cell Death Dis. 2019;10(2):40.
319. Pavlova NN, Zhu J, Thompson CB. The hallmarks of cancer metabolism: still emerging. Cell Metab. 2022;34(3):355–77.
320. Li X, Zhu H, Sun W, Yang X, Nie Q, Fang X. Role of glutamine and its metabolite ammonia in crosstalk of cancer-associated fibroblasts and cancer cells. Cancer Cell Int. 2021;21(1):479.
321. Bertero T, Oldham WM, Grasset EM, Bourget I, Boulter E, Pisano S, et al. Tumor-stroma mechanics coordinate amino acid availability to sustain Tumor Growth and Malignancy. Cell Metab. 2019;29(1):124–40. e10.
322. Buck MD, O'Sullivan D, Pearce EL. T cell metabolism drives immunity. J Exp Med. 2015;212(9):1345–60.
323. Huang M, Xiong D, Pan J, Zhang Q, Sei S, Shoemaker RH, et al. Targeting glutamine metabolism to Enhance Immunoprevention of EGFR-Driven Lung Cancer. Adv Sci (Weinh). 2022;9(26):e2105885.
324. Leone RD, Zhao L, Englert JM, Sun IM, Oh MH, Sun IH, et al. Glutamine blockade induces divergent metabolic programs to overcome tumor immune evasion. Science. 2019;366(6468):1013–21.
325. Oh MH, Sun IH, Zhao L, Leone RD, Sun IM, Xu W, et al. Targeting glutamine metabolism enhances tumor-specific immunity by modulating suppressive myeloid cells. J Clin Invest. 2020;130(7):3865–84.
326. Byun JK, Park M, Lee S, Yun JW, Lee J, Kim JS, et al. Inhibition of glutamine utilization synergizes with Immune checkpoint inhibitor to promote Antitumor Immunity. Mol Cell. 2020;80(4):592–e6068.
327. Geiger R, Rieckmann JC, Wolf T, Basso C, Feng Y, Fuhrer T, et al. L-Arginine modulates T cell metabolism and enhances survival and anti-tumor activity. Cell. 2016;167(3):829–42. e13.
328. Mossmann D, Müller C, Park S, Ryback B, Colombi M, Ritter N, et al. Arginine reprograms metabolism in liver cancer via RBM39. Cell. 2023;186(23):5068–e8323.
329. Caldwell RW, Rodriguez PC, Toque HA, Narayanan SP, Caldwell RB. Arginase: a multifaceted enzyme important in Health and Disease. Physiol Rev. 2018;98(2):641–65.
330. De Sanctis F, Lamolinara A, Boschi F, Musiu C, Caligola S, Trovato R, et al. Interrupting the nitrosative stress fuels tumor-specific cytotoxic T lymphocytes in pancreatic cancer. J Immunother Cancer. 2022;10(1):e003549.
331. Canale FP, Basso C, Antonini G, Perotti M, Li N, Sokolovska A, et al. Metabolic modulation of tumours with engineered bacteria for immunotherapy. Nature. 2021;598(7882):662–6.
332. Wang Z, Shao Y, Zhang H, Lu Y, Chen Y, Shen H, et al. Machine learning-based glycolysis-associated molecular classification reveals differences in prognosis, TME, and immunotherapy for colorectal cancer patients. Front Immunol. 2023;14:1181985.
333. Shi Z, Hu C, Zheng X, Sun C, Li Q. Feedback loop between hypoxia and energy metabolic reprogramming aggravates the radioresistance of cancer cells. Exp Hematol Oncol. 2024;13(1):55.
334. Sukumar M, Liu J, Ji Y, Subramanian M, Crompton JG, Yu Z, et al. Inhibiting glycolytic metabolism enhances CD8 + T cell memory and antitumor function. J Clin Invest. 2013;123(10):4479–88.
335. Guo C, You Z, Shi H, Sun Y, Du X, Palacios G, et al. SLC38A2 and glutamine signalling in cDC1s dictate anti-tumour immunity. Nature. 2023;620(7972):200–8.
336. Zhang X, Dang CV. Time to hit pause on mitochondria-targeting cancer therapies. Nat Med. 2023;29(1):29–30.
337. Li M, Thorne RF, Shi R, Zhang XD, Li J, Li J, et al. DDIT3 directs a dual mechanism to Balance Glycolysis and oxidative phosphorylation during glutamine deprivation. Adv Sci (Weinh). 2021;8(11):e2003732.
338. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366(26):2455–65.
339. Mariniello A, Nasti TH, Chang DY, Hashimoto M, Malik S, McManus DT, et al. Platinum-based chemotherapy attenuates the Effector response of CD8 T cells to concomitant PD-1 blockade. Clin Cancer Res. 2024;30(9):1833–45.
340. Wang G, Yao Y, Huang H, Zhou J, Ni C. Multiomics technologies for comprehensive tumor microenvironment analysis in triple-negative breast cancer under neoadjuvant chemotherapy. Front Oncol. 2023;13:1131259.
341. Walsh LA, Quail DF. Decoding the tumor microenvironment with spatial technologies. Nat Immunol. 2023;24(12):1982–93.
342. Stahl PL, Salmen F, Vickovic S, Lundmark A, Navarro JF, Magnusson J, et al. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. Science. 2016;353(6294):78–82.
343. Song AH, Jaume G, Williamson DFK, Lu MY, Vaidya A, Miller TR, et al. Artificial intelligence for digital and computational pathology. Nat Rev Bioeng. 2023;1(12):930–49.
344. van der Laak J, Litjens G, Ciompi F. Deep learning in histopathology: the path to the clinic. Nat Med. 2021;27(5):775–84.
345. Rabbani M, Kanevsky J, Kafi K, Chandelier F, Giles FJ. Role of artificial intelligence in the care of patients with nonsmall cell lung cancer. Eur J Clin Invest. 2018;48(4):e12901.
346. Koelzer VH, Sirinukunwattana K, Rittscher J, Mertz KD. Precision immunoprofiling by image analysis and artificial intelligence. Virchows Arch. 2019;474(4):511–22.
347. Saltz J, Gupta R, Hou L, Kurc T, Singh P, Nguyen V, et al. Spatial Organization and molecular correlation of Tumor-infiltrating lymphocytes using Deep Learning on Pathology images. Cell Rep. 2018;23(1):181–93. e7.
348. Zhao J, Fong A, Seow SV, Toh HC. Organoids as an enabler of Precision Immuno-Oncology. Cells. 2023;12(8):1165.

Publisher's Note