

## Review Article

# Emerging roles for tumor stroma in antigen presentation and anti-cancer immunity

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Advances in immunotherapy in the last decade have revolutionized treatment paradigms across multiple cancer diagnoses. However, only a minority of patients derive durable benefit and progress with traditional approaches, such as cancer vaccines, remains unsatisfactory. A key to overcoming these barriers resides with a deeper understanding of tumor antigen presentation and the complex and dynamic heterogeneity of tumor-infiltrating antigen-presenting cells (APCs). Reminiscent of the 'second touch' hypothesis proposed by Klaus Ley for CD4<sup>+</sup> T cell differentiation, the acquisition of full effector potential by lymph node-primed CD8<sup>+</sup> T cells requires a second round of co-stimulation at the site where the antigen originated, i.e. the tumor bed. The tumor stroma holds a prime role in this process by hosting specialized APC niches, apparently distinct from tertiary lymphoid structures, that support second antigenic touch encounters and CD8<sup>+</sup> T cell effector proliferation and differentiation. We propose that APC within second-touch niches become licensed for co-stimulation through stromal-derived instructive signals emulating embryonic or wound-healing provisional matrix remodeling. These immunostimulatory roles of stroma contrast with its widely accepted view as a physical and functional 'immune barrier'. Stromal control of antigen presentation makes evolutionary sense as the host stroma-tumor interface constitutes the prime line of homeostatic 'defense' against the emerging tumor. In this review, we outline how stroma-derived signals and cells regulate tumor antigen presentation and T-cell effector differentiation in the tumor bed. The re-definition of tumor stroma as immune rheostat rather than as inflexible immune barrier harbors significant untapped therapeutic opportunity.

## Tumor antigen presentation in the tumor microenvironment: main actors and their locations

Antigen processing and presentation by antigen-presenting cells (APCs) comprises the sentinel phase of the anti-tumor immune response and ushers the afferent arm of the cancer-immunity cycle [1] but increasingly significant roles for APC are recognized in the efferent arm of the cycle. Our understanding of the mechanisms regulating tumor antigen capture and presentation has evolved along the more general context of a deeper appreciation of the tumor microenvironment (TME) as a complex and dynamic entity [2]. Factors such as tumor-infiltrating immune cells, immunosuppressive cells (e.g. regulatory T cells), stromal cells, soluble factors (e.g. cytokines and chemokines), extracellular matrix, metabolites, hypoxia, pH in the TME shape the antigen capture and presentation process [3]. In this ever-changing milieu, the functions, and activities of professional and non-professional APCs play a pivotal role in anti-tumor immune responses [4].

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## Professional antigen-presenting cells

Dendritic cells (DCs), macrophages, and B cells are classically referred to as professional APCs because they express both Major histocompatibility class (MHC)-I and MHC-II surface molecules and are capable of presenting exogenous antigens, such as tumor antigens. Conventional DCs (cDCs) are the most potent professional APCs [5]. cDC precursors (pre-cDCs) develop from monocyte-dendritic cell precursors and commit to one of two distinct cDC lineages, cDC1 and cDC2 [6]. Multiple lines of evidence have demonstrated a pre-eminent role for cDC1 in tumor antigen presentation and anti-tumor immunity [7]. Mice lacking *Batf3*, a crucial transcription factor for cDC1 development, lose the capacity to reject immunogenic tumors [8]. Abundance of cDC1s in the TME is associated with T cell infiltration, overall survival in patients with cancer, as well as being predictive of response to immune checkpoint inhibitors [9]. Mouse cDC1s are efficient at taking up cell-associated antigen and dead cells via receptors such as CLEC9A, LY75, AXL, and HAVCR2 for eventual cross-presentation on MHC-I to CD8+ T cells [8]. The process of clearance of apoptotic cells by DC and macrophages, termed ‘efferocytosis’, may constitute a potentially underappreciated immune checkpoint and is comprehensively covered elsewhere [10]. An unexpected role for cDC1s in CD4+ T cell priming was recently demonstrated [11]. By presenting tumor antigens on MHC-II, cDC1s prime naïve CD4+ T cells and induce the expression of CD40 ligand on T cells which, in turn, license cDC1s via CD40 signaling. This signaling enhances priming of CD8+ T cells through mechanisms including induction of CD70 and potentially other co-stimulatory ligands and generate an effective anti-tumor CD8+ T cell response. cDC2s also uptake tumor antigens, are particularly efficient at presentation on MHC-II and induce superior CD4+ T cell proliferation [12]. cDC2 expressing interferon (IFN)-stimulated genes (ISG+ DCs) were recently shown to present intact tumor-derived peptide-MHC-I complexes (cross-dressing), activate CD8+ T cells and promote protective anti-tumor immunity in the absence of cDC1 [13]. These recent studies reinforce the concept that the functional dichotomy between cDC1s and cDC2s is not absolute, and cDC2s (and other cDCs) may acquire the ability to activate CD8+ T cells under certain inflammatory conditions.

Macrophages and B-cells are also professional APCs with established roles in tumor immunity. Tumor-associated macrophages (TAMs) are among the most abundant immune cells in the TME and perform diverse functions [14]. According to their functional differences, TAMs can be broadly categorized into M1 subtype (pro-inflammatory and anti-tumor) and M2 subtype (anti-inflammatory and pro-tumor). However, more recent high-resolution analyses have revealed enormous inter- and intra-tumor fluidity and heterogeneity of phenotypes and functional programs of intratumoral macrophages [15]. Moreover, the distinct origins of tissue-resident macrophages versus macrophages derived from infiltrating inflammatory monocytes are now better appreciated [16]. The functional plasticity of TAMs can influence antigen presentation and immune regulation within the TME.

B cells also make a critical contribution to adaptive immune responses [17]. In tumor tissues, B cells can be found in lymphoid aggregates, known as tertiary lymphoid structures (TLSs) [18] (see below). The TLS provides an area of intense B cell antigen presentation that can lead to optimal T cell activation and effector functions, as well as the generation of effector B cells, which can be further differentiated in either antibody-secreting plasma cells or memory B cells.

The functional characteristics of these APCs within the TME can be influenced by various factors, including tumor-derived signals, immunosuppressive factors, and the activities of other immune cells. These factors can impact the ability of APCs to effectively present antigens and initiate anti-tumor or pro-tumor immune responses. Understanding the interplay between APCs and the TME is crucial for developing strategies to enhance immune responses against cancer [19].

## Non-professional antigen-presenting cells

Whereas professional APCs constitutively express MHC Class-I and Class-II molecules and often scan tissues for exogenous material that could be associated with tissue damage and threat, non-professional APCs adopt antigen presentation functionality under certain conditions and can present antigen in the context of both MHC-I and -II. Non-professional APCs include stromal cells such as cancer-associated fibroblasts (CAFs), lymph node stromal cells, and endothelial cells (EC) [20]. Among these diverse cell types, antigen-presenting CAFs have gained significant interest, due to their major presence in the TME (detail in subsequent sections). Lymph node stromal cells are part of the secondary lymphoid tissue and regulate T cell activation against infections and tumor cells but also have key roles in homeostasis, maintaining peripheral tolerance by the induction of T cell anergy [20].

Endothelial cells are located along the inner lining of blood vessels and act as a barrier between blood and tissue. The most researched antigen-presenting endothelial cells are liver sinusoidal ECs (LSECs), which can cross-present soluble exogenous antigens in an equivalent way to DCs [20]. However, LSECs induce tolerance rather than stimulating T cell function. LSECs can also present exogenous antigens to CD4<sup>+</sup> cells on MHC-II molecules [21]. In a highly inflammatory microenvironment, antigen presentation by LSECs can generate a memory-like T cell population that can be reactivated upon antigen re-exposure [22,23].

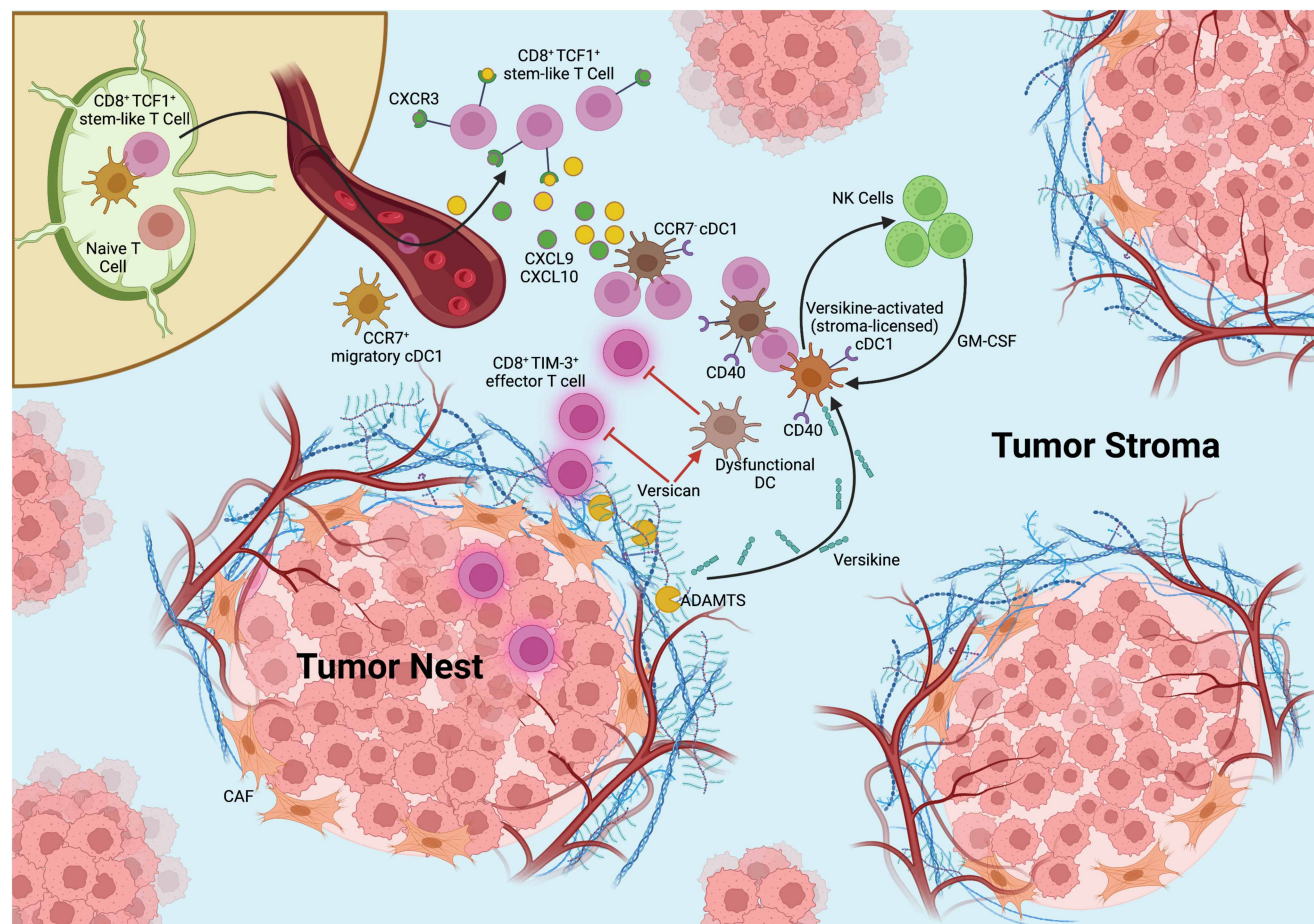
## Antigen presentation in the tumor bed versus the draining lymph node

cDC1s are recruited to the tumor bed through chemokines secreted by diverse cell sources, depending on the tumor type [24–26]. Inhibition of the recruitment of cDC1s can be an early mechanism to limit the development of anti-tumor immunity [27]. Still, cDC1s constitute the sparsest DC type in tumors. cDC1 density in the tumor is often influenced by its genetic make-up: for example, activation of the  $\beta$ -catenin signaling pathway prevents the recruitment of cDC1s to the tumor bed through interference with chemokine networks [24,26]. NK cells are essential for cDC1 recruitment into tumors through the secretion of DC-supporting differentiation/survival signals (e.g. FLT3L) or DC-chemoattractants, such as Chemokine (X–C Motif) Ligand 1 (XCL1) and Chemokine (C–C motif) Ligand 5 (CCL5) [4,7,25]. cDC1, uniquely among cDC subsets, express XCR1, the receptor for XCL1 [28,29]. The TME also actively produces cytokines that interfere with DC maturation, such as Interleukin (IL)-6, IL-10, and transforming growth factor (TGF)- $\beta$ , and thus promote the conversion of DCs into a tolerogenic phenotype [30–32]. A novel DC ‘state’ defined by the expression of immunoregulatory and maturation gene signature (mregDCs) was recently identified in human and mouse tumors [33]. This immunoregulatory program is associated with restrained cDC1 immunostimulatory function and limited T cell activation in the draining lymph nodes. In addition to defects in maturation, intratumoral cDC1s can exhibit impaired cross-presentation via elevated levels of oxidized lipid in DCs [34,35]. Thus, tumors can modulate their antigenicity by altering tumor-associated DC function.

Upon taking up dying tumor cells that release danger signals, including damage-associated molecular patterns (DAMPs), immature cDCs undergo a maturation process, defined by the up-regulated expression of co-stimulatory molecules, and migrate via lymphatic vessels to the tumor-draining lymph node (TDLN). There, migrating DCs present tumor antigens through the cross-presentation pathway for priming and activation of naïve CD8<sup>+</sup> T cells (immune priming) [36], whereas antigen is handed-over in part, to LN-resident DC. Activated T cells then leave the lymph nodes, migrate back to the TME, and exert their cytotoxic or helper functions against the tumor cells. The lymph nodes provide an environment replete with co-stimulatory molecules and cytokines conducive to immune priming, facilitating the expansion and differentiation of tumor-specific T cells. Additionally, the lymph node structures contain multiple cell types, including those of stromal origin like lymphatic endothelial cells, blood endothelial cells, and fibroblastic reticular cells [37]. These specialized subsets of cells are non-professional APCs and can fulfill crucial roles in regulating the T cell response (T-cell zone reticular cells) or B cell response (follicular DCs) [38]. The net outcome on T cell function is dependent on the type of co-regulatory signals provided by the APCs, including co-stimulatory signals (e.g. CD80/86) and co-inhibitory signals (e.g. PD-L1). Strong co-stimulatory signals license T cells to become fully activated, while lack of sufficient co-stimulatory signals leads to the induction of anergy and thus promotes immunological tolerance.

However, recent observations have led to an evolution of this model: while it is true that tumor-derived cDC1s migrate to the TDLN to present tumor antigens and prime T cells, the latter do not acquire a full effector program, as in the context of viral infection; instead they express markers of a stem-like state (e.g. TCF1) [39–41]. Stem-like Tcf1<sup>+</sup> PD1<sup>+</sup> Tim3<sup>−</sup> CD8<sup>+</sup> T cells egress the TDLN and reach the periphery of the tumor tissue, where they encounter DCs within specialized stromal niches (Figure 1). The interaction with these professional APCs further promotes T cell differentiation towards an effector Tcf1<sup>−</sup> PD1<sup>+</sup> Tim3<sup>+</sup> CD8<sup>+</sup> phenotype, that allows them to penetrate the tumor nest and initiate tumor cell killing [39]. While cDC1 may not be the exclusive APC type involved in these interactions, they appear to have a prime role in stimulating incoming primed stem-like CD8<sup>+</sup> T cells [42]. These findings are consistent with the ‘second touch’ hypothesis first formulated by Klaus Ley in the context of CD4<sup>+</sup> T cell differentiation [43]. The second touch hypothesis states that after initial antigen encounter in the draining LN (‘first-touch’), CD4<sup>+</sup> polarization towards various Th or Treg phenotypes requires *de novo* antigen encounter (second touch) in the tissue where the antigen originates.

The stromal APC niches supporting incoming stem-like T cell differentiation appear distinct from TLS [18]. The latter have been reported in many solid tumors and their presence has been correlated with prognosis and



**Figure 1. Specialized stromal APC niches support CD8+ T effector cell differentiation and anti-tumor immunity.**

Following LN-priming, stem-like CD8+ T cells receive a 'second touch' round of co-stimulation by APCs located within stromal niches along the tumor periphery. The resultant activation, proliferation and full effector differentiation of CD8+ T cells promotes 'T-cell inflammation' of the tumor nest. We propose that APC within these niches receive instructive signals from peri-tumoral stroma, reminiscent of embryonic or adult wound-healing provisional matrix remodeling. Stromal signals license APC for co-stimulation and activation of CD8+ T cells. One such stromal signal is provided by versikine, a proteolytic fragment of the large-matrix proteoglycan versican (VCAN). In contrast with versikine, full-length VCAN promotes a tolerogenic APC phenotype, thus establishing a regulatory loop between the parent macromolecule (VCAN) and its derivative 'matrikine'. Robust VCAN proteolysis is associated with T-cell inflammation across most solid and hematopoietic tumor types.

response to immunotherapy. They consist of ectopic follicles with distinct B and T cell compartmentalization and germinal centers. Mature TLS have been shown to foster affinity maturation to support the differentiation and functional specialization of anti-tumor plasma cells. Foundational events of TLS have been associated with specialized fibroblastic type cells secreting Chemokine (C-X-C motif) Ligand (CXCL) 13 or LIGHT [44,45]. In contrast, stromal APC niches resemble extrafollicular regions of lymphoid tissue where T cells reside [46]. In conclusion, the evolving understanding of the 'cancer-immunity cycle' reserves an essential role for antigen presentation not only in TDLN priming (afferent arm) but also in effector differentiation in the TME (efferent arm).

## Tumor stroma: immune barrier or immune rheostat?

The tumor stroma comprises a highly diverse and ever-changing composition of cellular and non-cellular components: CAFs, mesenchymal stromal cells (MSCs), immune cells, as well as a continuously remodeling extracellular matrix (ECM) and a unique vascular system that is specific to each cancer. This complex environment allows for the growth, invasion, and spread of tumors [47]. The understanding of stroma as an immunological interface has lagged behind the classic view of stroma as a mechanical boundary. However, it is increasingly

appreciated that stroma represents an ecosystem of immune activity that is malleable and pleiotropic. In the following section, we will highlight the dichotomous immunological activities of a cellular stromal actor (CAFs) and a non-cellular element (the large-matrix proteoglycan, VCAN).

## Dual roles of a cellular stromal actor: immunoregulatory CAFs versus immunostimulatory/antigen-presenting CAFs

CAFs have been classically thought of as pro-tumoral and immunosuppressive (for a comprehensive review of CAFs, see ref. [48]). Their activities in chemoattraction, stimulation and differentiation of regulatory immune cells are well-described [49–51]. Moreover, CAFs elaborate ECM macromolecules that can further enhance the stromal immunoregulatory function [52].

Two major classes of CAFs, both considered immunoregulatory, were first described in pancreatic cancer and subsequently validated in other tumor models [53]: myofibroblastic (myCAFs) and inflammatory (iCAFs). myCAFs are characterized by enhanced contractility and adhesion and are thought to represent the equivalent of wound-healing fibroblasts. They express smooth muscle actin (aSMA). They also secrete Th2 type cytokines IL-4, IL-13 and TGF $\beta$ . On the other hand, iCAFs express low levels of aSMA but are highly secretory. Their chemokine profile overlaps the senescence-associated secretory phenotype and includes multiple chemokine families including CXCL12, CCL2 and IL-6. iCAFs have been associated with the immunoregulatory polarization of several myeloid and lymphoid entities such as myeloid-derived suppressor cells (MDSC), type 2 macrophages and T-regs. myCAFs and iCAFs are not static entities but can interconvert [50].

The functions of CAFs in inflammation and innate immunity are well-described, in most instances pointing to a pro-tumoral role. CAFs in colorectal cancer have been found to be able to recruit monocytes and promote M2-macrophage polarization through an IL-8/CXCR2 axis and IL-6 secretion [54]. Furthermore, single-cell RNA sequencing has shown that tolerogenic TAM in the tumor are promoted by CAFs in triple negative breast cancer [55,56]. Conversely, an increase in CAFs was also found to characterize durable responses to checkpoint inhibition immunotherapy in melanoma, an effect partially mediated through TAMs and DCs [57]. CAFs can also directly modulate DCs in tumors. Cheng et al. found that CAFs in hepatocarcinoma (hCAFs) co-opted DC to adopt a STAT3-mediated tolerogenic phenotype via an IL-6 signaling pathway [58]. Both the CAFs and the STAT3-high DCs up-regulated IDO expression, which inhibited T cell expansion and cytotoxicity by catalyzing tryptophan depletion and kynurenine generation [58]. CAFs produce TGF $\beta$ , which promotes M2-polarized macrophages and a regulatory DC phenotype [59]. While less widely studied, CAFs have also been found to be able to directly impact B-cells. TGF $\beta$  has long been known to inhibit B-cell maturation and proliferation, and more recently has been shown to induce class switching to IgA-expressing B-cells [60–62]. Consequently, cytotoxic lymphocyte function was reduced both via reduced antigen presentation from fewer and immature B-cells, but also potentially from a direct effect of IgA-expressing B-cells [63].

Aside from directly acting on APCs, CAFs elaborate immunomodulatory ECM molecules that can influence APCs. For example, CAFs in breast cancer drastically up-regulate biglycan — an extracellular small leucine-rich proteoglycan (SLRPG) [64]. Biglycan is known to be able to act both on APCs of both the innate and adaptive immune system through stimulation of Toll like receptor (TLR) 2/4 receptors on macrophages and DCs [52,65]. CAFs have also been shown to be a significant source of hyaluronan (HA) in the TME [66]. HA with a molecular weight of less than or equal to 500 kDa is known to be able to activate macrophages and DCs, while high molecular-weight HA has diverse activities [66–68].

The paradigm of immunoregulatory CAFs has been disputed by the demonstration that in non-cancerous chronic inflammatory states, CAFs can be reprogrammed to support adaptive immunity: for example in patients with rheumatoid arthritis, inflammatory bowel disease or vitiligo, fibroblasts have been shown to secrete CXCL12 and CCL19 resulting in T cell infiltration of inflamed tissue [69,70]. High resolution single-cell transcriptomic analyses have revealed heterogeneity of tumor-infiltrating CAFs. myCAF deletion in pancreatic cancer was surprisingly associated with blunting of adaptive immunity [71]. Metabolically active CAFs have been associated with increased numbers of cytotoxic T cells and adaptive immune responses in both pancreatic and lung cancers [72,73]. In the latter, these CAFs secrete high amounts of T-cell chemoattractants CXCL11, CXCL12, CCL14 and CCL20 [73].

Further support to the concept of immunostimulatory CAFs has been provided by the demonstration of antigen-presenting CAFs (apCAFs) in pancreatic and other cancers [74]. In lung cancer, they were estimated to comprise >10% of all CAFs [75]. They often express the invariant chain CD74, cytokine IL-6 and

chemoattractants CXCL9 and CXCL10 [76–78]. In lung cancer, apCAFs express AT-II-specific surfactant genes as well as MHC-II, raising the possibility that they originate from AT-II cells [75]. Whether antigen-presenting abilities are shared by multiple CAF subsets in different contexts or only apCAFs is still debatable. It is clear however, that apCAFs can present MHC-II-restricted antigens on CD4+ T cells *in vivo* and CAF-specific deletion of MHC-II restricts antigen-specific CD4+ infiltration *in vivo* [75]. An immunostimulatory role for apCAFs was also suggested from studies in breast cancer [77,78] whereas in pancreatic cancer, Treg-inducing roles were described [79,80]. This pleiotropic role for apCAFs is not surprising as it mirrors hematopoietic APC functionalities that can be malleable according to the tissue and functional context. Therefore, cellular components of tumor stroma can fine-tune the immune response through context-specific immunomodulatory activities.

## Dual roles of a non-cellular stromal actor: the contrast between the immunoregulatory proteoglycan versican versus its immunostimulatory proteolytic fragment, versikine

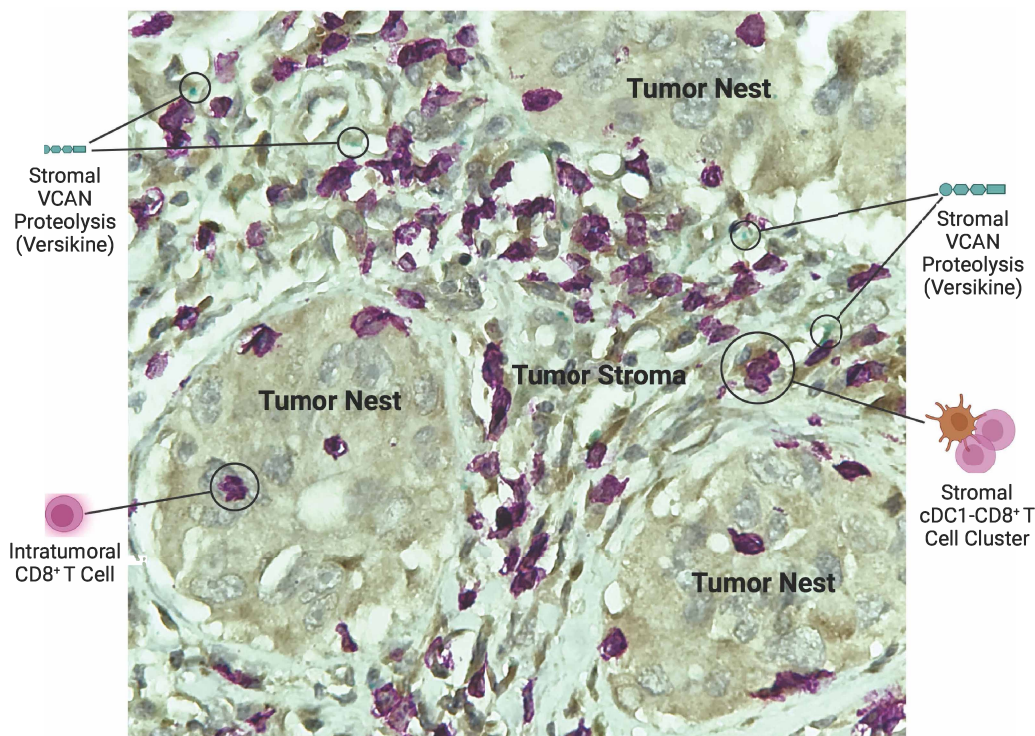
Versican (VCAN) is a large-matrix proteoglycan which is produced by a variety of cell types in the stroma and modified by a similarly diverse number of enzymes/proteases [81]. VCAN has been associated with TLR2/6-mediated TNF- $\alpha$  and other inflammatory cytokine secretion from TAMs and monocytes in both solid and hematological tumors [82,83]. These activities have been associated with the establishment of a pre-metastatic niche in carcinomas [84,85]. VCAN also has a role in adaptive anti-tumor immunity: it binds TLR2 on DCs [82], and renders them dysfunctional through the instigation of a tolerogenic IL-6 and IL-10 signaling loop [86].

Stromal VCAN is proteolyzed in T-cell inflamed tumors through ADAMTS proteases. The regulated proteolysis of VCAN at the Glu<sup>441</sup>–Ala<sup>442</sup> bond of the V1 isoform is associated with T-cell infiltration into the tumor bed and is predicted to release a bioactive fragment (‘matrikine’) [87], versikine [83,88–90] (Figure 2). Versikine promotes cDC1 abundance and activation [90]. The former effect appears to depend on an NK-dependent survival loop. Versikine-exposed cDC1 up-regulate CD40 and are hypersensitive to DNA-sensing through the STING pathway, the physiological pathway for innate sensing of cancers [91,92]. The fact that VCAN proteolysis is mostly confined to the tumor stroma [90], raises the hypothesis that versikine-licensed cDC1 interact with lymph-node-primed stem-like CD8+ T cells and versikine may license the stromal co-stimulation required for full effector CD8+ T cell differentiation (Figure 1). Indeed, consistent with this hypothesis, early evidence from the clinic suggests that tumors demonstrating VCAN proteolysis are more likely to respond to checkpoint inhibition immunotherapy [93].

VCAN proteolysis is a cardinal signaling modification in embryonic provisional matrix or the provisional matrix associated with wound healing [94–98]. In the embryo, VCAN proteolysis is essential for the development of the limb and circulatory systems [97]. Mice with targeted disruption of the Glu<sup>441</sup>–Ala<sup>442</sup> bond that generates versikine demonstrate developmental abnormalities [94,99]. In wound healing, disruption of the Glu<sup>441</sup>–Ala<sup>442</sup> bond, thus generating versikine from VCAN-V1, attenuates inflammation and promotes healing [94]. Similarly, in a model of inflammatory colitis, elimination of versikine reduces inflammation [100]. Thus, it appears that signals associated with morphogenesis in the embryo are co-opted in adult inflammation, wound healing and tumorigenesis (tumors being ‘wounds that do not heal’) to regulate the adaptive-innate immunity interface. In cancer, generation of versikine likely represents a homeostatic response against the developing tumor, that may promote immunosurveillance during the early stages of tumorigenesis [52]. Subsequent attenuation of versikine production from intact versican through proteolysis, an event associated with inflammation resolution in non-cancerous contexts, is exploited by the tumor to evade the immune response (immune ‘escape’) [52].

## Immunotherapeutic targeting of stroma: current approaches and challenges

Attempts to therapeutically alter stroma in cancer have targeted stromal cells as well as non-cellular stromal components. In the case of cell-directed therapies, the approaches have mirrored the rationale adopted when targeting tumor-promoting myeloid cells: the desired outcome is eliminating, reprogramming or neutralizing deleterious products (such as immunosuppressive cytokines). Earlier approaches such as agonistic CD40 antibodies have been used to indirectly remodel tumor stroma through activation of tumor-infiltrating myeloid cells and secretion of stroma-modifying enzymes [101]. Pathway-specific approaches to reverse tissue fibrosis



**Figure 2. Stromal versican proteolysis is associated with cDC1-CD8+ cross-talk and T-cell inflammation.**

In human T-cell-inflamed tumors, CD8+ T-cells penetrate into tumor nests, whereas cDC1 interact with CD8+ T-cells within adjacent stroma that recurrently displays site-specific proteolysis of the matrix proteoglycan versican (VCAN), an event associated with provisional matrix remodeling in embryonic development and adult wound healing [98]. Triple immunohistochemical staining of a human lung cancer biopsy [DPEAAE (versikine) = teal, XCR1 = brown, CD8 = purple]. XCR1 is a marker for cDC1. Magnification 400×.

while enhancing immune infiltration are highlighted by the targeted blockade of focal adhesion kinase (FAK). FAK is a nonreceptor protein tyrosine kinase which has been related to proliferation, invasion, angiogenesis and poor survival as well as drug resistance in several cancer malignancies [102]. In pancreatic ductal adenocarcinoma (PDAC), FAK activation antagonizes CD8<sup>+</sup> cytotoxic T lymphocyte infiltration through an increase in myeloid cell recruitment, pro-tumor polarization of macrophages and tissue fibrosis [103,104]. Consequently, FAK inhibitors (FAKi) potentiate the efficacy of immunotherapies in pre-clinical models [105], in part through decrease in tissue fibrosis and desmoplasia [103]. Clinical trials of FAK inhibitors in combination with PD-1 antagonist are already underway (ClinicalTrials.gov NCT02758587, NCT02546531). FAK inhibition could help overcome resistance to immunotherapies across a wide range of cancer types, but particularly those with an intense desmoplastic response.

More recently, direct attempts to target stromal fibroblasts have included cellular therapies against fibroblast activation protein (FAP) [106]. CAR-T cells targeting FAP have demonstrated promise in tumors with intense desmoplastic response, such as pancreatic cancer [107]. An interesting recent demonstration of the potential of this approach has been in the case of the hematopoietic cancer multiple myeloma, where dual targeting of tumor cells and stroma (through bi-specific CAR-T targeting myeloma cell tumor antigens and FAP, respectively) demonstrated superior effects compared with either single target alone [108]. More mechanistic, and perhaps more elegant approaches, include therapeutic targeting of specific receptors/signaling pathways that mediate interactions between immunosuppressive myeloid cells and stroma, such as LAIR receptors that recognize collagen [109].

As mentioned earlier, indiscriminatory targeting of CAFs can have undesired effects so caution is advised. Fortunately, the increasing focus on the therapeutic potential of targeting stroma is paralleled by the increasing reliability on single-cell analytical approaches that reveal the full heterogeneity and spectrum of the non-

hematopoietic stromal compartment (see ref. [110] for an excellent demonstration of the power of cutting-edge high-resolution technologies). Our ability to carry out precision-attack against tumor-promoting stromal actors while preserving tumor-countering stromal activities such as apCAFs will depend on a better understanding of their distinct modes and pathways of regulation through high resolution ‘-omic’ technologies. Alternatively, the focus can be on enhancing immunostimulatory CAFs or turning them into vehicles or platforms of vaccination. The latter could be achieved through nanotechnologies that can efficiently deliver molecular templates encoding tumor neoantigens into distinct subsets of antigen-presenting immunogenic mesenchymal cells [74]. The demonstration that mesenchymal stromal cells (MSC) can be converted into efficient vaccination platforms generates significant optimism to overcome manufacturing limitations associated with hematopoietic-derived antigen-presenting cells [111].

## Perspectives

- The tumor stroma has traditionally been considered as an immune barrier; however, this viewpoint is rapidly evolving. We propose that tumor stroma acts as an immune rheostat. Cellular stromal actors, e.g. CAFs, may exert context-specific pro- or anti-tumor effects. Moreover, stromal matrix-remodeling signals actively regulate the magnitude and quality of the adaptive anti-tumor immune response.
- Tumor stroma hosts specialized APC niches that are critical for anti-tumor immunity, through ‘second touch’ antigenic encounters promoting effector T cell proliferation and differentiation of lymph node-primed CD8<sup>+</sup> T cells. APC within stromal niches become licensed for co-stimulation through instructive stromal signals emulating embryonic or adult wound-healing provisional matrix remodeling.
- Provisional matrix-like stroma triggering T cell inflammation likely reflects the persistence of a host-derived anti-tumor homeostatic response. Transition into fibrotic stroma simulating embryonic matrix compaction and adult wound resolution is exploited by tumors to promote immune evasion.

## Competing Interests

A.P., A.C. and F.A. are named inventors on patents relating to the use of matrikines in immunotherapy.

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## Abbreviations

APCs, antigen-presenting cells; CAFs, cancer-associated fibroblasts; DCs, dendritic cells; ECM, extracellular matrix; FAK, focal adhesion kinase; FAP, fibroblast activation protein; HA, hyaluronan; IL, Interleukin; LSECs, liver sinusoidal ECs; MHC, major histocompatibility class; TAMs, Tumor-associated macrophages; TDLN, tumor-draining lymph node; TGF, transforming growth factor; TLSs, tertiary lymphoid structures; TME, tumor microenvironment; VCAN, versican; XCL1, X–C motif ligand 1.

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