

# Fibroblast heterogeneity in tumor micro-environment: Role in immunosuppression and new therapies

Rana Mhaidly, Fatima Mechta-Grigoriou

## ▶ To cite this version:

Rana Mhaidly, Fatima Mechta-Grigoriou. Fibroblast heterogeneity in tumor micro-environment: Role in immunosuppression and new therapies. Seminars in Immunology, 2020, 48, pp.101417 -. 10.1016/j.smim.2020.101417. hal-03493637

# HAL Id: hal-03493637 https://hal.science/hal-03493637

Submitted on 15 Dec 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Version of Record: https://www.sciencedirect.com/science/article/pii/S1044532320300336 Manuscript\_a7a2487b3a552fd0537de2a21b8720be

#### **Seminars in Immunology**

Special Issue: The Tumor Microenvironment: prognostic and theranostic impact. Recent advances and trends

## Fibroblast heterogeneity in Tumor Micro-environment: Role in Immunosuppression and New therapies

Rana Mhaidly<sup>1,2</sup> and Fatima Mechta-Grigoriou<sup>1, 2#</sup>

<sup>1</sup> Stress and Cancer Laboratory, Equipe labelisée Ligue Nationale Contre le Cancer, Institut Curie, PSL Research University, 26, rue d'Ulm, F-75005 Paris, France

<sup>2</sup> Inserm, U830, Paris, F-75005, France

# Corresponding author: Fatima Mechta-Grigoriou (ORCID Number: 0000-0002-3751-6989)
Phone: +33 (0)1 56 24 66 53; Fax: +33 (0)1 56 24 66 50; E-mail address: fatima.mechta-grigoriou@curie.fr

#### Abstract

In tumors, Cancer-Associated Fibroblasts (CAFs) constitute the most prominent component of the tumor microenvironment (TME). CAFs are heterogeneous and composed of different CAF subsets exerting distinct functions in tumors. Specific CAF subpopulations actively influence various aspects of tumor growth, including cancer cell survival and proliferation, angiogenesis, extracellular matrix (ECM) remodeling, metastatic spread and chemoresistance. During the past decade, some CAF subsets have also been shown to modulate anti-tumor immune response. Indeed, they can increase the content in regulatory T lymphocytes and inhibit the activity of effector and cytotoxic immune cells. These functions are mainly controlled by their constitutive secretion of cytokines, chemokines, growth factors and ECM proteins, either directly in the surrounding extracellular space or through micro-vesicles. Some CAFs also express key regulators of immune checkpoints. The different roles played by CAFs, both as immunosuppressor or as physical support for tumor cell progression, set them as promising targets for anti-tumor therapies. In this review, we describe the main current knowledge on CAFs heterogeneity and immunosuppressive microenvironment, as well as their potential therapeutic implications.

**Keywords:** cancer associated fibroblasts, CAF, immunosuppression, cancer, tumor microenvironment, stroma, T lymphocytes, NK, macrophages

#### 1. Generalities

It is now well-accepted that tumor progression is not strictly dependent on cancer cell genetic alterations or epigenetic modifications, but also controlled by components of the tumor microenvironment (TME). TME components are central to all stages of tumorigenesis and metastasis [1-4]. TME is composed of several cell types, such as fibroblasts, pericytes, immune and endothelial cells that are all able to interact with cancer cells in a dynamic way. Among cells present in the TME, cancer-associated fibroblasts (CAFs) represent one of the most abundant components that can contribute -at several levels- to the malignant phenotype [2-7]. In non-cancerous homeostatic conditions, fibroblasts are present at resident stage and represent an important sensor of tissue integrity. After a tissue damage signal, they differentiate into myofibroblasts and orchestrate tissue repair through synthesis of ECM components and crosstalk with immune cells [4]. Dysregulation of the physiological conditions during tumor development accompanied with a chronic inflammatory response drive fibroblast activation and their differentiation into myofibroblasts, i.e. fibroblasts with high constitutive expression of smooth-muscle  $\alpha$ -actin (SMA). CAFs are described to significantly increase tumor development by regulating several processes, including cancer cell proliferation, tumor cell invasion, angiogenesis and ECM remodeling. The abundance of SMA<sup>+</sup> CAFs in TME is associated with poor prognosis in multiple cancers [8-15]. Moreover, tumors with high stromal signatures are linked to therapy resistance and disease relapse [5, 16-19]. In addition to their role in controlling cancer cell behavior, CAFs emerge as central players in shaping the TME toward an immunosuppressive and growth-promoting phenotype, via increased production of immunosuppressive cytokines and enhanced expression of immune checkpoints [20-31]. Still, multiple findings showed that CAFs are not a homogenous cellular population. Indeed, several CAF subpopulations have been identified and have either tumor-promoting [25, 27, 31-36] or tumor-suppressive effects [37, 38]. Such heterogeneity might result from numerous causes, including the various cellular precursors of CAFs, the reciprocal interactions between CAFs and cancer cells or any other TME components, in addition to the cytokines, chemokines and growth factors secreted into the TME. Taking together, all these features highlight the importance of CAFs and define them as promising anti-cancer therapies, as a complement to existing treatments targeting cancer cells or boosting the immune system. In this review, we summarize recent advanced of CAFs heterogeneity in different cancer subtypes with a particular emphasis on the mechanisms involved in their immunosuppressive capacities. We will also highlight the potential therapeutic strategies for targeting CAFs in the field of cancer immunotherapy.

#### 2. CAF heterogeneity in TME

CAFs are major components of stromal cells that surround cancer cells. They provide not only a mechanical support to cancer cells but also control their proliferation, survival, metastasis and resistance to therapies [3-7, 25]. Although CAF origins in tumors remain unclear and might be diverse, CAF heterogeneity has been unveiled in the last years thanks to intense researches. In that sense, CAF subsets rather represent distinct cellular states than different cell types. Many studies have reported different origins of CAFs including resident tissue fibroblasts, mesenchymal stem cells from bone marrow or adipose tissue, as well as endothelial cells, epithelial cells, pericytes and adipocytes undergoing trans-differentiation into mesenchymal cells [34, 39-52]. Fibroblast differentiation into myofibroblasts (SMA+ fibroblasts) is driven by different mechanisms highlighted in distinct cancer subtypes. In breast carcinoma, contact between cancer cells and stromal cells promotes myofibroblastic CAF differentiation. The release of reactive oxygen species (ROS) by cancer cells plays a key role in the increase in myofibroblast content through the activation of various signaling pathways, including CXCL12/CXCR4, TGFβ and NOTCH signaling [2, 8, 52-54]. In mouse model of squamous cell carcinoma, the transition from normal fibroblasts to CAFs is mediated by two events, the suppression of p53 activity and down-modulation of the Notch effector Csl/Rbpj [55]. Interestingly, the induction of a pro-inflammatory gene signature in normal fibroblasts at early stage of squamous carcinogenesis promoted CAFs generation. Indeed, normal dermal fibroblasts can be educated by carcinoma cells to express proinflammatory genes, thereby promoting myofibroblastic differentiation [8, 56]. In addition, epigenetic modifications also contribute to fibroblasts reprogramming toward a pro-invasive phenotype, thereby promoting ECM remodeling and invasion of cancer cells [57-61]. Physical changes in ECM, including rigidity of collagen fibers and modulation of elasticity, govern fibroblast phenotypic modifications [62, 63 Arwert, 2020 #195, 64]. Mechanical stretching of normal fibroblasts in *in vitro* studies induces phenotypic changes similar to those of CAFs, increase their contractility and remodel TME to facilitate tumor invasion [65]. Furthermore, stretched fibroblasts produce ECM with a more organized and linearly aligned structure, with increased ability for redirecting co-cultured cancer cell migration [54, 65-75]. Tumor-stroma mechanics and ECM stiffening also coordinate non-essential amino-acid availability to sustain tumor malignancy [76]. In addition to the role of tumor cells in generating CAFs, signals from other cell types within the TME can also promote fibroblastic invasive functions. For instance, granulin-secretion by tumor-associated macrophages activated hepatic stellate

cells, driven their differentiation into myofibroblasts that secreted periostin, resulting in a fibrotic microenvironment. Disruption of macrophages recruitment, or genetic depletion of granulin reduced CAFs activation and prevented liver metastasis [77, 78]. Furthermore, recent data show a reciprocal crosstalk between regulatory T lymphocytes and CAF subsets in breast cancers [25, 31].

CAFs can be distinguished within the tumor by their morphological features and the lack of expression for epithelial, endothelial and immune cell markers. However, this is not sufficient to exclude other mesenchymal lineages, such as pericytes or adipocytes [4]. For these reasons, different markers, which are not or lowly expressed in normal fibroblasts compared to CAFs, were identified to better characterize activated CAFs, such as SMA, fibroblast activated protein (FAP), fibroblast specific protein-1 (FSP1, also known as S100A4), Integrin  $\beta$ 1 (CD29), platelet derived growth factor receptor  $\alpha$  or  $\beta$  (PDGFR $\alpha / \beta$ ) or podoplanin (PDPN) [79-84]. Still, these markers are not all regulated similarly or simultaneously in CAFs, thereby highlighting a strong degree of heterogeneity of these cells in TME. By combining the analysis of six CAF markers, four different CAF subpopulations (referred to as CAF-S1 to CAF-S4) were recently discovered in breast and ovarian cancers (Figure 1) [25, 27]. Both CAF-S1 (FAP<sup>High</sup> CD29<sup>Med</sup> SMA<sup>Med-High</sup> FSP1<sup>Med</sup> PDGFRβ<sup>Med-High</sup> CAV1<sup>Low</sup>) and CAF-S4 (FAP<sup>Neg-Low</sup> CD29<sup>High</sup> SMA<sup>High</sup> FSP1<sup>Low-Med</sup> PDGFR6<sup>Low-Med</sup> CAV1<sup>Low</sup>) subsets are detected at high level in aggressive breast cancer subtypes (HER2 and TN) and in metastatic lymph nodes, confirming that stromal myofibroblasts are associated with poor prognosis [25, 54]. Both CAF-S1 and CAF-S4 promote metastases through complementary mechanisms [54]. Moreover, accumulation of FAP<sup>high</sup> CAF-S1 subset in early luminal breast cancers is associated with distant relapse [85]. In contrast, the CAF-S2 subset (CD29<sup>Low</sup> FAP<sup>Neg</sup> FSP1<sup>Neg-Low</sup>  $\alpha$ -SMA<sup>Neg</sup> PDGFR $\beta$ <sup>Neg</sup> CAV1<sup>Neg</sup>) is enriched in luminal breast cancer subtype and CAF-S3 fibroblasts (CD29^{Med} FAP^{Neg} FSP1^{Med-high} \alpha-SMA^{Neg} PDGFR $\beta^{Med}$  CAV1<sup>Low</sup>) accumulate in healthy tissues [25, 54, 85]. In all tumors and invaded lymph nodes, FAP<sup>high</sup> CAF-S1 fibroblasts are defined by ECM, adhesion and wound-healing signatures, and FAP<sup>Neg</sup> CD29<sup>High</sup> CAF-S4 are characterized by a perivascular contractile signature[25, 27, 54, 85]. Several recent studies from bulk or single cell data on CAF from human cancers and mouse models confirmed the existence of ECM-rich (CAF-S1) and contractile (CAF-S4) sub-populations [36, 86-90]. By applying specific CAF-S1 and CAF-S4 molecular signatures on publicly available single cell data from lung cancer [91] and head and neck cancer [92], the existence of these two major CAF-S1/CAF-S4 myofibroblastic

subpopulations was validated in different cancer types [31]. Thus, these data indicate that both CAF-S1 (ECM/adhesion/wound) and CAF-S4 (perivascular/contractile) myofibroblastic cells can be detected in distinct cancer types and across species.

Among the FAP<sup>High</sup> CAF (CAF-S1) subpopulation, two distinct subsets were recently identified in pancreatic adenocarcinoma, exhibiting either a matrix-producing myofibroblastic phenotype termed myCAF or an immunomodulatory secretome, inflammatory CAFs named iCAF [33]. More precisely, CAFs most proximal to the cancer cells exhibit a myofibroblastic myCAF phenotype with high expression of SMA. More distal CAFs express high levels of IL-6 and are defined as iCAFs [33, 87]. Furthermore, two FAP<sup>+</sup> mesenchymal subsets were also identified on the basis of PDPN expression [26]. Although both subsets express ECM molecules, FAP<sup>+</sup> PDPN<sup>+</sup> fibroblasts exhibit higher expression of genes associated with TGFB signaling and fibrosis than FAP<sup>+</sup> PDPN<sup>-</sup> cells. These observations demonstrated that the FAP<sup>High</sup> CAF-S1 subpopulation could be -by itself- heterogenous. Several laboratories have addressed this question by performing single cell analysis from FAP<sup>High</sup> CAF cells [31, 87-90]. All these studies demonstrated the existence of the iCAF/myCAF subpopulations in the different cancer types analyzed. Interestingly, the large number of FAP<sup>High</sup> CAF (CAF-S1) fibroblasts recently sequenced at single cell levels in [31] reached -to our knowledge- an unprecedented resolution of this subpopulation. This study identified 8 different FAP<sup>High</sup> CAF clusters with 3 clusters belonging to the iCAF subgroup and 5 clusters to the myCAF subgroup (Figure 1). By dissecting the most prominent pathways and gene signature specific of each cluster, these 8 clusters can be characterized as followed: ECM proteins (ecmmyCAF), detoxification pathway (detox-iCAF), interleukin-signaling (IL-iCAF), TGFβdependent pathway (TGF $\beta$ -myCAF), wound-healing signaling (wound-myCAF), IFN $\gamma$ - and IFNαβ-related pathway (IFNγ-iCAF, IFNαβ-myCAF) and acto-myosin-signaling (actomyCAF) [31]. Interestingly, the ecm-myCAF specific signature contains the LRRC15 gene that has been recently identified in pancreatic cancer [90]. Moreover, the IFNy-iCAF cluster express high levels of CD74, encoding Major Histocompatibility Class (MHC) II invariant chain, which characterizes the antigen-presenting CAF ("apCAF") recently identified in pancreatic cancer [89]. Finally, the existence of the most abundant FAP<sup>High</sup> CAF clusters has been validated in head and neck squamous cell carcinoma and in non-small cell lung cancer [31].

In conclusion, these recent data demonstrate that CAF heterogeneity in cancer has been until now largely under-estimated. Still, a common spectrum of CAF subpopulations is emerging, with both CAF-S1 (FAP<sup>High</sup> CD29<sup>Med</sup> SMA<sup>Med-High</sup>; ECM/adhesion/wound signatures) and CAF-S4 (FAP<sup>Neg-Low</sup> CD29<sup>High</sup> SMA<sup>High</sup>; perivascular/contractile signatures) myofibroblastic CAF detected in distinct cancer types and across species. Moreover, among FAP<sup>High</sup> CAF-S1 subpopulation, the iCAF/myCAF subsets are validated in all cancers analyzed until now. Finally, these iCAF and myCAF subsets can be -by themselves-subdivided into different clusters identified as detox-iCAF, IL-iCAF, IFN $\gamma$ -iCAF and ecmmyCAF, TGF $\beta$ -myCAF, wound-myCAF and IFN $\alpha\beta$ -myCAF, respectively. These FAP<sup>High</sup> CAF clusters have been detected in various cancer types, thereby demonstrating their validity and their potential important roles in tumors.

#### 3. Role of CAFs in anti-tumor immune response

As discussed above, it is now well established that CAFs exhibit a phenotypic diversity and functional heterogeneity that define their role within the tumor [7, 8, 20, 25, 26, 31, 37, 38, 54, 87, 90, 93-95]. The pro-tumorigenic functions of CAFs could be attributed either to their ability to produce pro-survival factors, which directly and positively impact tumor progression by enhancing cancer cell proliferation, survival and metastasis, or to their role in regulating antitumor immune response by inducing an immunosuppressive microenvironment [8, 21-25, 27, 31, 87, 96-102]. The immunomodulatory functions of these stromal cells can affect both innate and adaptive antitumor immune response (Figure 2). This can be either direct via the secretion of cytokines and chemokines such as IL-6, CXCL1, CXCL12, CCL2, which can retain suppressive immune subsets and counteract effector functions of activated immune cells, or indirect via the remodeling of the ECM protein network, forming a physical barrier for immune cell entry [29, 30, 62, 65, 70, 103-108].

#### 3.1. CAF-dependent regulation of the adaptive anti-tumor immune response

#### 3.1a- Role of CAF subsets in adaptive anti-tumor response

The pleiotropic immunomodulatory functions of CAFs can interfere with the adaptive antitumor immune responses at different stages of cancer progression (Figure 2). In this context, multiple studies have confirmed the presence of functionally heterogeneous CAFs subpopulations within the same tumors. For example, in breast cancer, among FAP<sup>High</sup> CAF, it was shown that PDPN<sup>+</sup> fibroblasts suppress T cell proliferation in a nitric-oxide-dependent manner, whereas PDPN<sup>-</sup> cells are unable to do so [26]. Importantly, a multistep mechanism unraveling FAP<sup>High</sup> CAF-mediated immunosuppressive activity has been recently highlighted in breast and ovarian cancer [25, 27]. By secreting CXCL12 -through regulation by mir-200 family members in ovarian cancer [27]-, FAP<sup>High</sup> CAF attract CD4<sup>+</sup> CD25<sup>+</sup> T lymphocytes and retain them at their surface by high expression of OX40L/TNFSF4, PD-L2/PDCD1LG2 and JAM2 in several cancer types[25, 27]. Moreover, FAP<sup>High</sup> CAF increase CD4<sup>+</sup> T cell survival and their differentiation into CD25<sup>High</sup> FOXP3<sup>High</sup> functional Tregs through B7H3/CD276, CD73/NT5E, and DPP4 [25, 27]. As detailed above, among FAP<sup>High</sup> CAF, recent single cell analyses reveal the heterogeneity within this population [31, 33, 87-90]. On line with the identification of different clusters among FAP<sup>High</sup> CAF, recent data demonstrated that only specific clusters, in particular those characterized by ECM accumulation, woundhealing signature and TGFβ-signaling, are associated with an immunosuppressive environment [31, 89, 90]. Consistent with this correlation between the abundance of FAP<sup>High</sup> ecm-myCAF and TGFβ-myCAF cellular clusters with the content in FOXP3<sup>+</sup> T lymphocytes in breast cancer, *in vitro* functional assays showed that ecm-myCAF are able to recruit FOXP3<sup>high</sup> Tregs and to increase PD-1 and CTLA-4 protein levels at their surface [31]. Interestingly, accumulation of ecm-myCAF, wound-myCAF and TGFβ-myCAF is correlated with resistance to immunotherapy in melanoma and non-small cell lung cancer patients [31].

#### 3.1b- Role of CAF subsets on immune checkpoint expression

Regulating immune checkpoint expression emerged as an important process by which CAFs directly modulate T cell function. An interesting point was recently raised showing that CAFs from breast, ovarian, lung, pancreas and colon cancer express programmed death ligand-1/2 PD-L1 and/or PD-L2 [25, 27, 109-112], in particular in the FAP<sup>High</sup> CAF subset [25, 27]. These ligands bind to PD-1 receptor expressed by T cells and inhibit T cell activity [25, 110-112]. PD-L1 and PD-L2 ligands can be upregulated by IFN- $\gamma$  highly secreted by activated T cells, thereby impairing their function [109]. Moreover, fibroblasts isolated from melanoma patients' biopsies secrete IL-1 $\alpha/\beta$  that could directly suppress CD8<sup>+</sup> T cell proliferation and function via upregulation of PD-L1 expression at their surface [113], suggesting that IL-1 $\alpha/\beta$  clinical blockade might synergize with immunotherapeutic interventions and benefit melanoma patients. Similarly, CAFs isolated from pancreatic cancer also promote expression of immune checkpoint inhibitors TIM-3, PD-1, CTLA-4 and LAG-3 at the surface of proliferating T cells, via the activity of CAF-derived PGE2 [112]. Finally,  $\alpha$ -SMA<sup>+</sup> CAF are positively correlated with PD-L1 expression by tumor cells in melanoma and colorectal carcinoma [114]. Mechanistically, CXCL5 secreted by CAFs promotes PD-L1

expression at the surface of tumor cells which in turn abolished T cell anti-tumor immune response [114].

#### 3.1c- Role of metabolism in CAF-mediated immunosuppressive action

Another suggested mechanism by which CAFs impair T cell proliferation and function is depending on the production of metabolic reprogramming factors [115-120]. First, in contrast to normal fibroblasts, CAFs can use aerobic glycolysis as a source of energy, thus producing high levels of pyruvate and lactate that modify T cell polarization, reducing the percentage of antitumoral helper T cell (T<sub>H</sub>1) subset and increasing Treg proliferation and function [119-123]. Another example of immunosuppressive metabolites secreted by CAFs is mediated by the immunomodulatory indoleamine 2,3-dioxygenase (IDO) enzyme that acts as a T-cell inhibitory effector [124, 125]. Indeed, IDO facilitates the conversion of tryptophan to kynurenine and production of downstream metabolites that inhibit T-cell proliferation and function [124, 125]. Similarly, CAF-dependent expression of Arginase 2, which hydrolyzes arginine to ornithine and urea, reduces Arginine content in TME, which is indispensable for T cell functions [126, 127]. Consistent with this key function, the abundance of Arginase 2expressing stromal cells is associated with poor clinical outcome in pancreatic cancer [126, 127]. Furthermore, CAFs are a major source of PGE2, which plays an important role in promoting tumor growth, angiogenesis and resistance to established cancer therapies [128-133]. In addition, it was shown that PGE2 drives immunosuppression by multiple mechanisms, either by shifting the balance from anti-tumor T<sub>H</sub>1 immune response toward immunosuppressive T<sub>H</sub>2 response, or by suppressing T cell cytotoxic activity and promoting Treg function [134-139]. Moreover, COX2, which catalyzes PGE2 production, is highly expressed in CAF and results in an exacerbated Treg recruitment in tumors [139-142]. CAFs also produce galectins, known as S-type lectins, carbohydrate-binding proteins that mediate cell-cell or cell-ECM communications [143, 144]. Galectin-1 induces T cell apoptosis by binding on CD7 and CD45 on T cell surface. In addition, galectin-1 is overrepresented in Tregs and contributes to their immunosuppressive activity [145-147]. Furthermore, mesenchymal stromal cells isolated from cervical tumors showed elevated expression levels of CD39 and CD73 as compared to normal fibroblasts [148]. This expression reduces T cell activity and proliferation, through the generation of a large amount of adenosine, a wellknown immunosuppressive molecule produced by the hydrolysis of ATP, ADP and AMP [148]. On line with this observation, FAP<sup>High</sup> CAF express high levels of CD73, which promotes immunosuppression by increasing Tregs in breast and ovarian cancer [25, 27].

#### 3.1d- TGF $\beta$ -dependent signaling pathway in CAF-mediated immunosuppression

One cellular cluster recently identified among FAP<sup>High</sup> CAF by single cell analysis in breast cancer is characterized by TGFB signaling and is directly involved in immunosuppression and resistance to immunotherapies [31]. Indeed, some CAFs, in particular some FAP<sup>High</sup> CAFs, secrete high levels of TGFB ligands that act on both CD8<sup>+</sup> and CD4<sup>+</sup> T cells [25, 31, 149-156]. TGF $\beta$  was shown to promote cell death of effector CD8<sup>+</sup> T lymphocytes by inhibiting the expression of the pro-survival protein BCL2 [111]. It attenuates the acquisition of effector function by memory CD8<sup>+</sup> T cells, preventing their access to cancer cells and providing a barrier for the therapeutic cancer vaccines [104, 157]. TGFβ also alters CD8<sup>+</sup> T cell function by inhibiting the expression of key genes involved in their cytotoxic activity (perforin, granzymes A and B, FAS ligand and IFNy). For instance, repression of granzyme B and IFNy transcription is mediated by the binding of TGFB-activated SMAD and ATF1 transcription factors on their promoters. Neutralization of systemic TGF $\beta$  in mice enables tumor clearance with restoration of cytotoxic gene expression in activated CD8<sup>+</sup> T cells [158]. In head and neck cancer, α-SMA<sup>+</sup> FAP<sup>+</sup> CAFs inhibit the proliferation of CD8<sup>+</sup> T cells and promote the recruitment of CD4<sup>+</sup> CD25<sup>+</sup> T cells by secreting TGF<sup>β</sup> and IL6 [22]. TGF<sup>β</sup> stimulates also class switch recombination, converting IgM expressing-B cells to IgA expressing-cells which present regulatory activity. In hepatocellular carcinoma, these IgA<sup>+</sup> cells interfere with activation of cytotoxic CD8<sup>+</sup> T lymphocytes inhibiting thus, tumor-directed immune response [159, 160]. In addition to TGF $\beta$ , it was shown in a mouse model of colon cancer that CAFs release high levels of IL6 which in turn, decrease CD8<sup>+</sup> T cells infiltration within the tumor and increased Foxp3<sup>+</sup> Treg activation, effect attenuated by treatment with IL6 blocking antibodies [161].

Consistent with their immunosuppressive functions, TGF $\beta$  signature in CAFs is associated with poor response to immunotherapies in metastatic urothelial and colon cancer [28, 162], as well as in non-small cell lung cancer and in melanoma patients [31]. This can occur particularly in patients with tumors presenting an exclusion of CD8<sup>+</sup> T cells from the tumor [28, 162] and an accumulation in the collagen-rich peritumoral stroma, in line with ECM-enriched FAP<sup>High</sup> CAFs [31]. Interestingly, co-administration of TGF $\beta$  and anti-PD-L1 blocking antibodies reduces TGF $\beta$  signaling in stromal cells, facilitates T cell penetration, reduces tumor progression and improves patient prognosis [28, 163-166].

In addition to CD8<sup>+</sup> T cell and Tregs, CAFs have the potential ability to influence CD4<sup>+</sup> helper T cell phenotype, switching them from anti-tumoral to pro-tumoral cells. Indeed, CD4<sup>+</sup> T cells can be classified into multiple sub-lineages, based on their functions and the profile of cytokine production. In general, T<sub>H</sub>2-mediated immunity is considered tumor promoting, as it activates M2-like macrophages function and inhibits cell-mediated tumor killing. Many findings emerged from murine models of cancer, showed that targeting of specific signaling molecules in CAFs resulted in reduction of tumor growth, accompanied by a shift in the T cell response[20, 167-174]. For example, in a mouse model of breast cancer, elimination of FAP<sup>+</sup> CAFs by a DNA vaccine targeting FAP results in a shift of the immune population from a T<sub>H</sub>2 to a T<sub>H</sub>1 phenotype [167]. This shift was associated with an increased expression of IL-2, an increase of CD8<sup>+</sup> T cell population and a diminished recruitment of tumor-associated macrophages (TAM) and Tregs. Furthermore, targeting of CAF-derived Chitinase-3-like 1 (Chi3L1) has a similar effect in another transplantable model of breast cancer [175]. Indeed, it was demonstrated that Chi3L1 is highly upregulated in CAFs from mammary tumors and pulmonary metastases of transgenic mice. Genetic ablation of Chi3L1 in fibroblasts attenuated tumor growth, macrophage recruitment and reprogramming to an M2 phenotype and enhanced CD8<sup>+</sup> and CD4<sup>+</sup> T cells accumulation within the tumor [175]. Consistent with these findings, CHI3L1 has been shown to promote tumor progression through TGF $\beta$  pathway and pro-inflammatory signals and to be associated with poor prognosis in many cancers [176-182].

In conclusion, through distinct molecular mechanisms, CAFs modulate T celldependent antitumor immune response at multiple levels, i.e. by recruiting CD4<sup>+</sup> CD25<sup>+</sup> Tregs, by switching a  $T_{\rm H}1$  to a  $T_{\rm H}2$  phenotype, and by inhibiting CD8<sup>+</sup> cytotoxic activity.

#### 3.2. CAF regulation of the innate anti-tumor immune response

Besides the adaptive immunomodulatory functions, myofibroblastic CAFs also interfere with the innate immune response at different levels (Figure 2). First, it has been shown that CAFs affect the differentiation of tumor associated macrophages (TAMs) [183-185]. TAMs derive from infiltrating monocytes or resident macrophages and represent a major component within tumors. They are involved in various tumor-promoting tasks including pro-inflammatory signaling, enhancement of angiogenesis, metastasis and therapy resistance. CAFs actively polarize resident macrophages toward a pro-tumoral phenotype via the secretion of IL-6 and granulocyte-macrophage colony stimulating factor (GM-CSF) [186-188]. In addition, the glycoprotein CHI3L1, highly expressed by CAFs, enhances TAM recruitment and their

differentiation into pro-tumoral M2 [175]. CAFs also increase the recruitment of monocyte in tumors through CXCL12 delivery and promote their differentiation toward the M2 phenotype [185, 186, 189-194]. This interaction between CAFs and M2 enhances tumor progression and metastasis formation. CAFs abundancy is correlated with a higher number of TAMs in human breast cancer, correlation associated with a high Ki-67 proliferation index and a high tumor volume [193].

Similarly as the phenotypic macrophages (M1/M2), the concept of immune cell polarization has also been extended to neutrophils, named Tumors-associated neutrophils (TANs). N1 neutrophils show an anti-tumor phenotype with their capability of efficiently killing microorganisms and tumor cells, while N2 neutrophils exhibit a pro-tumor phenotype [195, 196]. CAF-mediated TGF $\beta$  signaling redirects TAN differentiation toward the N2 pro-tumorigenic phenotype. Conversely, TGF $\beta$  blockade attenuates tumor growth via TAN polarization to an anti-tumor N1 phenotype, thereby providing additive strategies for cancer therapies [197]. Moreover, CAF-derived IL6 increases survival, proliferation and activation of TAN in a STAT3-dependent manner and induces PD-L1 expression in TAN [198], indicating that IL6-STAT3-PD-L1 signaling cascade by CAF could provide novel targets for therapies [198-204].

Another mechanism by which CAFs trigger anti-tumor immune responses is by affecting the function of dendritic cells (DC), the most important population of antigen presenting cells, needed to activate T lymphocytes. As a major source of TGF $\beta$ , CAF mediate downregulation of MHC class II molecules and of CD40, CD80 and CD86 co-stimulatory molecules at the surface of DC, thus inhibiting their antigen presenting function and their ability to activate cytotoxic T cell responses [205]. Moreover, CAF-produced IDO facilitates the generation of regulatory DCs, characterized by low expression of costimulatory molecules, high levels of suppressive cytokines, impaired T-cell proliferation and enhanced Treg expansion [206-208]. Furthermore, proinflammatory cytokines (such as TNF $\alpha$  and IL-1 $\beta$ ) released by pancreatic tumor cells activate CAFs and facilitate secretion of thymic stromal lymphopoietin (TSLP) by CAFs [209]. TSLP induces activation/maturation of tumor-resident DCs, that migrate to the draining LNs where they activate CD4<sup>+</sup> T<sub>H</sub>2 cells and exert tumor-promoting functions [209].

Another immune cell population affected by CAFs immunosurveillance is mast cells. It is a heterogeneous population of immune cells, widely distributed throughout all tissues. Upon activation, mast cells release a large spectrum of cytokines and inflammatory molecules that modulate tumor initiation and progression. Interestingly, in pancreatic tumors, stellate cells (known as precursors of CAFs) activate mast cells and facilitate their degranulation and cytokine release. As a result, activated mast cells secrete IL-13 and tryptase, in the tumor microenvironment, leading to stromal cell proliferation, and favoring tumor growth [210]. Of note, activated mast cells not only increase tumor progression but also alter anti-tumor immune response. Indeed, mast cells mobilize myeloid-derived stem cells (MDSCs) infiltration into tumors and induce production of IL-17 by MDSCs; MDSCs-derived IL-17 indirectly attract Tregs and enhance their suppressive function [211]. MDSCs are a group of heterogeneous cells that are derived from bone marrow and have a remarkable ability to suppress immune cell responses. Multiple studies have shown a direct interaction between CAFs and MDSCs. Indeed, CAFs, isolated from hepatocellular carcinoma attract monocytes through the CXCL12/CXCR4 pathway and induce their differentiation into MDSCs through IL-6 mediated STAT3 activation [198, 212]. This interaction subsequently impairs T cell proliferation and alters their cytotoxic function. Moreover, FAP<sup>+</sup> CAF, as a major source of CCL2, promote tumor growth via the recruitment of MDSCs in a mouse model of hepatic cancer and in lung squamous cell carcinoma [24, 213].

Finally, CAFs can also affect the activity of major innate effector cells, Natural Killer (NK) cells, which orchestrate early immune response through their cytotoxic activity [214]. CAF-produced TGF $\beta$  ligands are important regulators of NK immune function. Indeed, TGF $\beta$ is well known to downregulate the expression of NKp30 and NKG2D activating receptors, thereby decreasing NK cell cytotoxic activity. In addition, TGF<sup>β</sup> induces the expression of the miR-183, which bind and repress DNAX activating protein 12 (DAP12), a signal adaptor for lytic function in NK cells [215-217]. TGFB also inhibits CD16-mediated IFN-y production and antibody-dependent cellular cytotoxicity, an effect that reduces T<sub>H</sub>1 immune cell differentiation [218, 219]. Moreover, melanoma and colorectal carcinoma-derived CAFs sharply interfere with NK cell functions including cytotoxicity and cytokine production by repressing the expression of several NK activating receptors including NKp44, NKp30 and DNAx accessory molecule 1 (DNAM-1), as well as the production of cytolytic granules containing perforin and Granzyme B [220, 221]. This modulation is mediated by the secretion of prostaglandin E2 (PGE2), leading to an attenuated cytotoxic activity of NK cells [220, 221]. In addition, elevated secretion of matrix metalloproteinases (MMPs) by CAFs in melanoma reduces the expression of MHC class I chain-related protein A and B (MICA/B) at the surface of tumor cells and consequently decrease NKG2D-dependent cytotoxic activity of NK cells [222]. CAFs also secrete adenosine that restricts NK cell function and immune protection against tumor development [223]. Finally, studies using endometrial cancer cells showed that CAF promoted NK dysfunction through contact dependent mechanisms involving downregulation, at the surface of CAFs, of poliovirus receptor (PVR/CD155), an important NK cell ligand [224]. Better understanding of the mechanisms through which CAFs manipulate NK cell activation will be needed as NK cells are attractive targets for novel adoptive cell immunotherapy [223, 225-227].

In conclusion, due to their ability to secrete immunomodulatory cytokines, chemokines or soluble factors, CAFs favor the recruitment of innate immune cells, such as monocytes or neutrophils and their acquisition of anti-inflammatory phenotypes like M2 macrophages or N2 neutrophils, but can also affect the cytotoxic activity of NK cells

#### 3.3. Indirect effect of CAFs on anti-tumor immune response

One of the hallmarks of CAFs is the excessive production of extracellular matrix components, which results in a remodeling of the ECM, and affects tumor cell behavior [68, 228]. Increased ECM rigidity resulting from thickening of collagen fibers provides cues favoring migration, attachment, survival and proliferation of cancer cells [229]. It also restricts the access of immune cells to cancer cells, serving as a physical barrier [230]. Multiple findings have shown that CAF-mediated ECM remodeling and fibrosis contributed to the formation of an immunosuppressed microenvironment by multiple mechanisms [230].

In this regard, in pancreatic adenocarcinoma, dense collagen networks represented a physical barrier, inhibiting the migration of activated T cells, which can alter the development of T-cell-based immunotherapies [231-233]. Similar results were observed in human lung tumors, showing that the presence of dense matrix fibers surrounding the tumor islets prevents T cell infiltration [103].

In addition, ECM remodeling, mediated by CAFs acts directly on tumor-specific CD8<sup>+</sup> T cells and F4/80 macrophages, either by reducing the number of CD8<sup>+</sup> T cells within the tumor or by functionally reprogramming F4/80 macrophages differentiation toward M2 antiinflammatory phenotype [234-238]. Thus, CAF-mediated ECM remodeling and fibrosis contribute to the formation of an immunosuppressed microenvironment that promotes tumor growth and metastasis formation.

#### 4. CAFs: a promising target for cancer therapy

In situation in which the tumor stroma promotes cancer progression and induces resistance to anticancer therapies, the development of new therapies targeting the stroma could have curative outcomes. In addition, the recent characterization of CAFs, including identification of new CAFs subsets and their functional diversities, brings CAF-targeting therapies as promising tools for cancer treatment (Figure 3) [95, 106, 239].

#### 4.1. CAF depletion via cell surface markers

As discussed above, FAP and SMA represent two cell surface markers for activated myofibroblasts. Based on this, targeting immunosuppressive CAFs using these markers was largely investigated in pre-clinical studies. Thus, targeting FAP by both genetic deletion or pharmacological inhibition inhibit tumor growth in two different mouse model of lung and colon cancer [240]. Similarly, conditional depletion of FAP<sup>+</sup> stromal cells in a mouse model of pancreatic cancer re-sensitizes cancer cells to anti-PD-1 and anti-CTLA-4 immunotherapies [241]. Multiple other approaches were developed to target FAP<sup>+</sup> CAFs, including an oral DNA vaccine targeting FAP [242], or an administration of a novel anti-FAP monoclonal antibody (FAP5-DM1) [243] that bound shared epitopes of mouse, human and monkey FAP and showed an excellent efficacy with a complete tumor regression without significant toxicity. In another metastatic breast cancer model, depletion of FAP<sup>+</sup> stromal cells by FAP-targeting immunotoxin ar AP-PE38 alters the secretion of various growth factors, cytokines and matrix metalloproteinases by myofibroblasts and suppresses tumor growth [244]. Interestingly, genetic depletion of FAP<sup>+</sup> CAFs is associated with increased CD8<sup>+</sup> T cells infiltration in Lewis lung carcinoma and pancreatic cancer models [168, 173]. Similarly, administration of an oral mouse DNA vaccine targeting FAP increases CD8<sup>+</sup> T cell infiltration and improves the intra-tumoral uptake of chemotherapeutic drugs in a model of colon cancer [242].

Based on these promising preclinical data, a phase I trial was started with a monoclonal antibody recognizing FAP (F19) in patients with colorectal cancer and small cell lung cancer [245, 246]. The results showed that this antibody was administrated safety to patients even after repeated infusions and was associated with tumor regression. However, it failed in patients with metastatic colorectal cancer [247]. Interestingly, CAR therapy was also used to target FAP<sup>+</sup> CAFs in the tumor stroma. For this purpose, T cells are modified to express a FAP-specific chimeric antigen receptor and transferred to an established A549 lung cancer model. These CAR T cells promote a specific immune attack against FAP<sup>+</sup> stromal cells inducing a significant decrease in tumor growth [169]. Similarly, adoptive transfer of

FAP-CAR T cells also decreases tumor vascular density and restraines growth of desmoplastic human lung cancer xenografts and syngeneic murine pancreatic cancers [248]. However, it is important to note that an opposite result came from another study and showed that depletion of FAP<sup>+</sup> stromal cells led to loss of muscle mass and anemia confirming that using FAP as a universal target antigen to deplete stromal cells should still be investigated [249]. A recent study investigated the use of a bispecific antibody (RO6874281) consisting of an interleukin 2 variant domain that binds to IL-2 receptor and stimulates a local immune response by activating cytotoxic NK cells and lymphocytes T, associated to a FAP-specific domain targeting CAFs. This component showed an acceptable safety profile and displayed monotherapy activity in different tumors [250]. These promising results drive the development of multiple clinical trials consisting to combine RO6874281 together with immunotherapies [251-254]. Moreover, a second bispecific protein was developed in order to target simultaneously the T cell costimulatory receptor 4-1BB and FAP+ stromal cells (FAP-4-1BB). Interestingly, treatment with this compound in a colorectal cancer-bearing rhesus monkey, decreased tumor growth and enhanced accumulation and activation of intratumoral CD8+ T cells [255, 256]

SMA was also used to target myofibroblastic CAFs. In a mouse model of breast cancer, docetaxel conjugate nanoparticules that target SMA<sup>+</sup> stromal cells suppress metastasis formation [257]. In parallel, in a mouse model of pancreatic cancer crossed with SMA thymidine kinase transgenic mice, depletion of SMA<sup>+</sup> myofibroblasts results unexpectedly in more invasive tumors with enhanced hypoxia, EMT induction and diminished animal survival [37]. Moreover, suppressed immune surveillance with increased CD4<sup>+</sup> Foxp3<sup>+</sup> Tregs was observed in myofibroblast-depleted mice accompanied with a resistance to anti-CTLA-4 immunotherapy [38]. These results suggested that targeting CAFs using surface markers requires careful consideration and might be more challenging than initially thought.

More recently, two cell-surface molecules, CD10 and GPR77, were defined as new markers for a CAFs subset correlated with chemoresistance and poor survival in multiple cohorts of breast and lung cancer patients. Mechanistically, CD10<sup>+</sup>GPR77<sup>+</sup> CAFs promote tumor formation and chemoresistance by providing a survival niche for cancer stem cells. Moreover, targeting these CAFs with a neutralizing anti-GPR77 antibody abolishes tumor formation and restored tumor chemosensitivity [35].

#### 4.2. Restoration of quiescent fibroblasts phenotype

As mentioned above, CAFs can result from activation of resident fibroblasts. Thus, it was considered to revert CAF activated state to a quiescent state in order to reduce tumor growth. In pancreatic tissue, resident fibroblasts store retinol that is lost upon activation. Restoring retinol level in CAFs in a mouse model of pancreatic adenocarcinoma resets fibroblasts to an inactive state and results in enhanced tumor necrosis, increased vascularity and diminished hypoxia with a reduction in tumor size [258]. In a parallel study, treatment of pancreatic stromal cells with Vitamin D markedly reduces markers of inflammation and fibrosis [259]. Interestingly, Vitamin D-treated fibroblasts get back to quiescent cell features, resulting in stromal remodeling that facilitates the delivery of chemotherapeutic agents within tumors [260]. Interestingly, a phase II clinical trial of concomitant treatment with PD-1 inhibitor and Vitamin D analog is now underway [261]

#### 4.3. Targeting downstream effectors and activation signaling of CAFs

Because depletion of CAFs or reversing their activated phenotype remains challenging, other therapeutic options have been proposed, such as targeting CAF downstream effectors. As described above, the IL6/JAK/STAT3 signaling pathway increases proliferation, survival and metastasis of tumor cells, accompanied with a strong suppression of anti-tumor immune response [262]. IL6 also induces production of pro-inflammatory and pro-angiogenic factors, including IL-1 $\beta$ , IL8 and multiple chemokines that act on both immune and non-immune cells. Moreover, IL6 activates the JAK/STAT3 pathway, which negatively regulates the cytotoxic activity of NK and T cells. Agents targeting this pathway already received FDA approval for the treatment of inflammatory pathologies and myeloproliferative neoplasms, and their efficacy was also approved in patients with hematopoietic malignancies and solid tumors, as they reduce tumor growth and increase sensitivity to chemotherapies [263, 264]. The mTOR/4E-BP1 regulatory pathway is up-regulated in primary cultures of SMA<sup>+</sup> CAFs isolated from human PDAC [265]. These cells abundantly expressed the somatostatin receptor 1, which is known to mediate inactivation of mTOR/4E-BP1 pathway. Thus, treatment with the somatostatin analog SOM230 in a murine xenograft model of pancreatic cancer down-regulates CAF-secreted molecules, including IL6, and abrogates CAF-mediated tumor growth [265, 266]. Importantly, combination of SOM230 with gemcitabine, the standard chemotherapy for pancreatic cancer, reduces tumor growth and facilitates the delivery of chemotherapeutic agents within the tumor. These results highlight a novel promising anti-tumor therapy indirectly targeting pancreatic cancer cell invasion through pharmacological inhibition of stromal cells [265, 266]. Additionally, PDGF signaling has a

key role in the recruitment and phenotypic changes of fibroblasts upon activation [25, 27, 52, 87, 267]. Interestingly, inhibition of stromal PDGF receptors in a mouse model of human cervical cancer reduces proliferation and angiogenesis, through a mechanism involving the suppression of expression of the angiogenic factor fibroblast growth factor FGF-2 and epithelial cell growth factor FGF-7 [267, 268].

FAP<sup>+</sup> CAF are a major source of CXCL12 secretion, which has a crucial role in immunosuppression and in resistance to anti-PD-1 and anti-CTLA-4 immunotherapies in pancreas, breast and ovarian cancer [20, 25, 27]. Moreover, administration of AMD3100, an inhibitor of CXCR4, the receptor of CXCL12, provides therapeutic interest in HER2 breast cancer patients [269]. Moreover, AMD3100 enhances T cell accumulation and acts synergistically with anti-PD-L1 antibody to diminish cancer progression in a pancreatic cancer mouse model [20]. Similarly, other proteins secreted by CAFs were also targeted in order to restrain their immunosuppressive capacities, such as TGFB. In many preclinical studies, TGFβ-targeting agents have shown potent anti-tumor effects. For example, disruption of TGF $\beta$  signaling using the TGF $\beta$  receptor kinase inhibitor (LY2157299) or a TGF $\beta$ neutralizing antibody (1D11) reduces the formation of bone metastasis from breast tumors [270, 271]. In addition, trihydroxyphenolic compounds were identified as potent blockers of TGF-\beta1 in *in vivo* models of pulmonary fibrosis and lung cancer metastasis. Remarkably, trihydroxyphenolics functional effects require the presence of active lysyl oxidase-like 2 (LOXL2), only produced by fibroblasts and cancer cells. Administration of these compounds resulted in potent blockade of pathological collagen accumulation in vivo without toxicity [272]. In addition, the use of Tranilast (a known suppressor of fibroblast proliferation and TGFβ secretion) in mice bearing E.G7 lymphoma, LLC1 Lewis lung cancer, or B16F1 melanoma decreases infiltration of immune suppressor cells, such as Tregs and MDSC, and activates CD8<sup>+</sup> T lymphocytes [170]. Similarly, Artemisinin, identified as an inhibitor of TGFβ signaling that reverts CAFs from an activated to an inactivated state, suppresses CAFsinduced breast cancer growth and metastasis by blocking the interaction between tumor cells and their microenvironment [273]. In addition, TGF $\beta$  inhibitor is able to synergize with the anti-OX40 antibody to elicit a potent anti-tumor effect, associated with an overall accumulation of CD4<sup>+</sup> and CD8<sup>+</sup> effector T lymphocytes [274]. Beside the highly positive data observed in animal models, clinical trials with some agents targeting TGF $\beta$  have been recently initiated [275-277], although TGF $\beta$  can act as a tumor suppressor at early tumor stages and TGF $\beta$  inhibitors are not cytotoxic [274].

#### 4.4. Targeting CAF-derived ECM proteins

As mentioned earlier, fibroblasts activation results in ECM remodeling through production of several components, including collagen fibers and ECM-degrading proteases, such as MMPs. This remodeling increases tissue stiffness and matrix rigidity and serves as a physical barrier that inhibits the access of anti-tumor immune cells and impedes the delivery of therapeutic drugs [29, 30, 65, 70, 103, 104] . Thus, targeting the ECM proteins or degrading ECM could be a new therapeutic option. Hyaluronan, produced by CAFs, is considered as the primary matrix molecule responsible for vessel compression, in a collagen-dependent manner [278, 279] . In this sense, the angiotensin inhibitor losartan, reduces stromal collagen and hyaluronan production and decreases the expression of profibrotic signals (TGF\$1, CCN2) [280]. Consequently, losartan increases vascular perfusion and improves drug and oxygen delivery to tumors, thereby potentiating chemotherapy in breast and pancreatic cancer models. Similarly, an enzymatic depletion of hyaluronic acid using a clinically recombinant PH20 (PEGPH20) facilitates the intra-tumoral penetration hyaluronidase of standard chemotherapeutic agents [281, 282]. This component, in combination with gemcitabine shows therapeutic benefit in patients with advanced pancreatic cancer [283]. In addition, as pancreatic adenocarcinoma is poorly vascularized, the administration of IPI-926, a specific inhibitor of Hedgehog signaling pathway, reduces tumor-associated stromal tissue, enhances intra-tumor vascular density and increases concentration of gemcitabine [284-286]. Furthermore, CAF-mediated ECM remodeling cannot be achieved without the production of MMPs that influence ECM degradation and facilitate cell migration. Despite promising preclinical data supporting the blockage of MMPs as a treatment for cancer, phase III clinical trials failed [287-290]. This was due principally to inadequate clinical trial design and poor knowledge of the complexity of the MMPs. Still, better and more specific inhibitors have been developed but are not yet ready for clinical use [291, 292].

Collectively, drugs that target stromal CAFs have emerged as an important option to improve anti-cancer therapies and drug resistance. However, the functional complexity and heterogeneity of CAF sub-populations within the same tumor might be taken into consideration to avoid off-target side effects.

#### **5-** Conclusion

It is nowadays commonly accepted that CAF are one of the major components of tumors that mainly support different steps of cancer progression. However, the precise definition of CAF heterogeneity and CAF subpopulations, with the complex identification of specific markers and specific CAF subpopulation functions, remain the most challenging aspects in the study of CAFs. Recently, rapid advances have been made in the molecular characterization of CAFs. This provides a better understanding on the mechanisms underlying CAF subsetsmediated tumor promotion and immunosuppression. Subsequently, these recent knowledges have facilitated the development of novel therapeutic strategies for cancer treatment. On the whole, the aforementioned anti-CAF therapies are designed to target immunosuppressive CAFs functions, which promote cancer development. Nevertheless, other CAFs subtypes have also been recently identified, which display tumor-suppressive capacities and can hold therapeutic potential.

#### Acknowledgements

R.M. is supported by the Institut National du Cancer (INCa) and the Simone and Cino del Duca Foundation. The Stress and Cancer laboratory is supported by grants from the Ligue Nationale Contre le Cancer (*Labelisation*), Inserm (PC201317), Institut Curie (Incentive and Cooperative Program Tumor Micro-environment PIC TME/T-MEGA, PIC3i CAFi), ICGex (ANR-10-EQPX-03), SIRIC (INCa-DGOS-4654), INCa (STROMAE INCa-DGOS-9963, CaLYS INCa-11692, INCa-DGOS-Inserm-12554) and the Foundation "Chercher et Trouver". F.M-G acknowledges both the Association « Le cancer du sein, Parlons-en / Ruban Rose », the Simone and Cino del Duca Foundation for attribution of their "Grand Prix". F.M-G is very grateful to all her funders for providing support throughout the years.

### **Declaration of interest**

F.M-G. received research support from Innate-Pharma, Roche and Bristol-Myers-Squibb (BMS). R.M. declares no potential conflict of interest.

#### Legends of figures

#### Figure 1: Schematic representation of CAF heterogeneity

CAFs represent a heterogeneous cellular population within the tumor microenvironment. By combining the analysis of six CAF markers, four different CAFs populations (referred as CAF-S1 to CAF-S4) were discovered recently in breast and ovarian cancers. While CAF-S1 present an immunosuppressive function, CAF-S4 promote metastasis formation. Later on, single cell analysis allows the identification of 8 different clusters within the CAF-S1 subset with 3 clusters belonging to the iCAF subgroup and 5 clusters to the myCAF subgroup. Both ecm-myCAF and TGFβ-myCAF exhibit an immunosuppressive function and are associated with resistance to immunotherapy.

#### Figure 2: CAFs-mediated immunosuppression

Due to their secretion of cytokines, chemokines, or other soluble factors, CAFs shape the immune response within the tumors toward a pro-tumorigenic microenvironment, by affecting both innate and adaptive immune cells

#### **Figure 3: Principal strategies for CAF-directed anticancer therapy:**

Multiple approaches have been developed to target CAFs for cancer treatment. CAFs can be directly depleted by targeting CAFs surface markers (FAP, GPR77). This depletion is mediated by either transgenic technologies or CAR-T cell therapies. Moreover, blocking secreted cytokines and specific effectors of CAFs (IL-6, TGF-b, CXCL12) or other growth factor pathways (PDGFR, mTOR) can be also used to inhibit the activation and function of CAFs.

### References

- 1. Quail, D.F. and J.A. Joyce, *Microenvironmental regulation of tumor progression and metastasis*. Nat Med, 2013. **19**(11): p. 1423-37.
- 2. Costa, A., A. Scholer-Dahirel, and F. Mechta-Grigoriou, *The role of reactive oxygen species and metabolism on cancer cells and their microenvironment*. Semin Cancer Biol, 2014. **25**: p. 23-32.
- 3. Pure, E. and R. Blomberg, *Pro-tumorigenic roles of fibroblast activation protein in cancer: back to the basics*. Oncogene, 2018. **37**(32): p. 4343-4357.
- 4. Sahai, E., et al., *A framework for advancing our understanding of cancer-associated fibroblasts.* Nat Rev Cancer, 2020. **20**(3): p. 174-186.
- 5. Correia, A.L. and M.J. Bissell, *The tumor microenvironment is a dominant force in multidrug resistance*. Drug Resist Updat, 2012. **15**(1-2): p. 39-49.
- 6. Hanahan, D. and L.M. Coussens, *Accessories to the crime: functions of cells recruited to the tumor microenvironment*. Cancer Cell, 2012. **21**(3): p. 309-22.
- Kalluri, R., *The biology and function of fibroblasts in cancer*. Nat Rev Cancer, 2016. 16(9): p. 582-98.
- 8. Toullec, A., et al., *Oxidative stress promotes myofibroblast differentiation and tumour spreading*. EMBO Mol Med, 2010. **2**(6): p. 211-30.
- 9. Saigusa, S., et al., *Cancer-associated fibroblasts correlate with poor prognosis in rectal cancer after chemoradiotherapy*. Int J Oncol, 2011. **38**(3): p. 655-63.
- 10. Becht, E., et al., *Immune and Stromal Classification of Colorectal Cancer Is Associated with Molecular Subtypes and Relevant for Precision Immunotherapy*. Clin Cancer Res, 2016. **22**(16): p. 4057-66.
- 11. Qin, X., et al., *Cancer-associated Fibroblast-derived IL-6 Promotes Head and Neck Cancer Progression via the Osteopontin-NF-kappa B Signaling Pathway.* Theranostics, 2018. **8**(4): p. 921-940.
- 12. Sun, Q., et al., *The impact of cancer-associated fibroblasts on major hallmarks of pancreatic cancer*. Theranostics, 2018. **8**(18): p. 5072-5087.
- 13. Alcaraz, J., et al., *Stromal markers of activated tumor associated fibroblasts predict poor survival and are associated with necrosis in non-small cell lung cancer*. Lung Cancer, 2019. **135**: p. 151-160.
- 14. Mizutani, Y., et al., *Meflin-Positive Cancer-Associated Fibroblasts Inhibit Pancreatic Carcinogenesis.* Cancer Res, 2019. **79**(20): p. 5367-5381.
- 15. Heichler, C., et al., *STAT3 activation through IL-6/IL-11 in cancer-associated fibroblasts promotes colorectal tumour development and correlates with poor prognosis.* Gut, 2020. **69**(7): p. 1269-1282.
- 16. Finak, G., et al., *Stromal gene expression predicts clinical outcome in breast cancer*. Nat Med, 2008. **14**(5): p. 518-27.
- 17. Calon, A., et al., *Stromal gene expression defines poor-prognosis subtypes in colorectal cancer*. Nat Genet, 2015. **47**(4): p. 320-9.
- 18. Liu, L., et al., Stromal Myofibroblasts Are Associated with Poor Prognosis in Solid Cancers: A Meta-Analysis of Published Studies. PLoS One, 2016. **11**(7): p. e0159947.
- 19. Fiori, M.E., et al., *Cancer-associated fibroblasts as abettors of tumor progression at the crossroads of EMT and therapy resistance.* Mol Cancer, 2019. **18**(1): p. 70.
- 20. Feig, C., 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): p. 20212-7.

- 21. Denton, A.E., et al., *Fibroblastic reticular cells of the lymph node are required for retention of resting but not activated CD8+ T cells.* Proc Natl Acad Sci U S A, 2014. **111**(33): p. 12139-44.
- 22. Takahashi, H., et al., *Immunosuppressive activity of cancer-associated fibroblasts in head and neck squamous cell carcinoma*. Cancer Immunol Immunother, 2015. **64**(11): p. 1407-17.
- 23. Ruhland, M.K., et al., *Stromal senescence establishes an immunosuppressive microenvironment that drives tumorigenesis.* Nat Commun, 2016. **7**: p. 11762.
- 24. Yang, X., et al., FAP Promotes Immunosuppression by Cancer-Associated Fibroblasts in the Tumor Microenvironment via STAT3-CCL2 Signaling. Cancer Res, 2016. **76**(14): p. 4124-35.
- 25. Costa, A., et al., *Fibroblast Heterogeneity and Immunosuppressive Environment in Human Breast Cancer*. Cancer Cell, 2018. **33**(3): p. 463-479 e10.
- 26. Cremasco, V., et al., *FAP Delineates Heterogeneous and Functionally Divergent Stromal Cells in Immune-Excluded Breast Tumors.* Cancer Immunol Res, 2018. **6**(12): p. 1472-1485.
- 27. Givel, A.M., et al., *miR200-regulated CXCL12beta promotes fibroblast heterogeneity and immunosuppression in ovarian cancers.* Nat Commun, 2018. **9**(1): p. 1056.
- 28. Mariathasan, S., et al., *TGFbeta attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells.* Nature, 2018. **554**(7693): p. 544-548.
- 29. Ziani, L., S. Chouaib, and J. Thiery, *Alteration of the Antitumor Immune Response by Cancer-Associated Fibroblasts*. Front Immunol, 2018. **9**: p. 414.
- 30. De Jaeghere, E.A., H.G. Denys, and O. De Wever, *Fibroblasts Fuel Immune Escape in the Tumor Microenvironment*. Trends Cancer, 2019. **5**(11): p. 704-723.
- 31. Kieffer, Y., et al., *Single-cell analysis reveals fibroblast clusters linked to immunotherapy resistance in cancer*. Cancer Discov, 2020.
- 32. Ohlund, D., E. Elyada, and D. Tuveson, *Fibroblast heterogeneity in the cancer wound*. J Exp Med, 2014. **211**(8): p. 1503-23.
- 33. Ohlund, D., et al., *Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer.* J Exp Med, 2017. **214**(3): p. 579-596.
- 34. Raz, Y., et al., *Bone marrow-derived fibroblasts are a functionally distinct stromal cell population in breast cancer.* J Exp Med, 2018. **215**(12): p. 3075-3093.
- 35. Su, S., et al., *CD10(+)GPR77(+)* Cancer-Associated Fibroblasts Promote Cancer Formation and Chemoresistance by Sustaining Cancer Stemness. Cell, 2018. **172**(4): p. 841-856 e16.
- 36. Neuzillet, C., et al., *Inter- and intra-tumoural heterogeneity in cancer-associated fibroblasts of human pancreatic ductal adenocarcinoma*. J Pathol, 2019. **248**(1): p. 51-65.
- 37. Ozdemir, B.C., et al., *Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival.* Cancer Cell, 2014. **25**(6): p. 719-34.
- 38. Rhim, A.D., et al., *Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma.* Cancer Cell, 2014. **25**(6): p. 735-47.
- 39. Cano, A., et al., *The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression.* Nat Cell Biol, 2000. **2**(2): p. 76-83.
- 40. Thiery, J.P. and J.P. Sleeman, *Complex networks orchestrate epithelial-mesenchymal transitions*. Nat Rev Mol Cell Biol, 2006. **7**(2): p. 131-42.
- 41. Zeisberg, E.M., et al., *Discovery of endothelial to mesenchymal transition as a source for carcinoma-associated fibroblasts.* Cancer Res, 2007. **67**(21): p. 10123-8.

- 42. Potenta, S., E. Zeisberg, and R. Kalluri, *The role of endothelial-to-mesenchymal transition in cancer progression*. Br J Cancer, 2008. **99**(9): p. 1375-9.
- 43. Quante, M., et al., *Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth.* Cancer Cell, 2011. **19**(2): p. 257-72.
- 44. Kidd, S., et al., Origins of the tumor microenvironment: quantitative assessment of adipose-derived and bone marrow-derived stroma. PLoS One, 2012. 7(2): p. e30563.
- 45. Bochet, L., et al., Adipocyte-derived fibroblasts promote tumor progression and contribute to the desmoplastic reaction in breast cancer. Cancer Res, 2013. **73**(18): p. 5657-68.
- 46. Cortez, E., P. Roswall, and K. Pietras, *Functional subsets of mesenchymal cell types in the tumor microenvironment*. Semin Cancer Biol, 2014. **25**: p. 3-9.
- 47. Sharon, Y., et al., *Tumor-derived osteopontin reprograms normal mammary fibroblasts to promote inflammation and tumor growth in breast cancer.* Cancer Res, 2015. **75**(6): p. 963-73.
- 48. Hosaka, K., et al., *Pericyte-fibroblast transition promotes tumor growth and metastasis.* Proc Natl Acad Sci U S A, 2016. **113**(38): p. E5618-27.
- 49. Morel, A.P., et al., *A stemness-related ZEB1-MSRB3 axis governs cellular pliancy and breast cancer genome stability.* Nat Med, 2017. **23**(5): p. 568-578.
- 50. Strong, A.L., et al., Obesity Enhances the Conversion of Adipose-Derived Stromal/Stem Cells into Carcinoma-Associated Fibroblast Leading to Cancer Cell Proliferation and Progression to an Invasive Phenotype. Stem Cells Int, 2017. 2017: p. 9216502.
- 51. Di Carlo, S.E. and L. Peduto, *The perivascular origin of pathological fibroblasts*. J Clin Invest, 2018. **128**(1): p. 54-63.
- 52. Strell, C., et al., *Impact of Epithelial-Stromal Interactions on Peritumoral Fibroblasts in Ductal Carcinoma in Situ.* J Natl Cancer Inst, 2019. **111**(9): p. 983-995.
- 53. Gentric, G., V. Mieulet, and F. Mechta-Grigoriou, *Heterogeneity in Cancer Metabolism: New Concepts in an Old Field.* Antioxid Redox Signal, 2017. **26**(9): p. 462-485.
- 54. Pelon, F., et al., *Cancer-associated fibroblast heterogeneity in axillary lymph nodes drives metastases in breast cancer through complementary mechanisms.* Nat Commun, 2020. **11**(1): p. 404.
- 55. Procopio, M.G., et al., *Combined CSL and p53 downregulation promotes cancerassociated fibroblast activation*. Nat Cell Biol, 2015. **17**(9): p. 1193-204.
- 56. Erez, N., et al., *Cancer-Associated Fibroblasts Are Activated in Incipient Neoplasia to Orchestrate Tumor-Promoting Inflammation in an NF-kappaB-Dependent Manner.* Cancer Cell, 2010. **17**(2): p. 135-47.
- 57. Jiang, L., et al., *Global hypomethylation of genomic DNA in cancer-associated myofibroblasts.* Cancer Res, 2008. **68**(23): p. 9900-8.
- 58. Albrengues, J., et al., *LIF mediates proinvasive activation of stromal fibroblasts in cancer*. Cell Rep, 2014. **7**(5): p. 1664-1678.
- 59. Albrengues, J., et al., *Epigenetic switch drives the conversion of fibroblasts into proinvasive cancer-associated fibroblasts*. Nat Commun, 2015. **6**: p. 10204.
- 60. Du, H. and G. Che, *Genetic alterations and epigenetic alterations of cancerassociated fibroblasts.* Oncol Lett, 2017. **13**(1): p. 3-12.
- 61. Mishra, R., et al., *Epigenetic changes in fibroblasts drive cancer metabolism and differentiation*. Endocr Relat Cancer, 2019. **26**(12): p. R673-R688.

- 62. Calvo, F., et al., *Mechanotransduction and YAP-dependent matrix remodelling is required for the generation and maintenance of cancer-associated fibroblasts.* Nat Cell Biol, 2013. **15**(6): p. 637-46.
- 63. Avery, D., et al., *Extracellular matrix directs phenotypic heterogeneity of activated fibroblasts*. Matrix Biol, 2018. **67**: p. 90-106.
- 64. Arwert, E.N., et al., *STING and IRF3 in stromal fibroblasts enable sensing of genomic stress in cancer cells to undermine oncolytic viral therapy.* Nat Cell Biol, 2020. **22**(7): p. 758-766.
- 65. Goetz, J.G., et al., *Biomechanical remodeling of the microenvironment by stromal caveolin-1 favors tumor invasion and metastasis.* Cell, 2011. **146**(1): p. 148-63.
- 66. Tomasek, J.J., et al., *Myofibroblasts and mechano-regulation of connective tissue remodelling*. Nat Rev Mol Cell Biol, 2002. **3**(5): p. 349-63.
- 67. Ao, M., et al., *Stretching fibroblasts remodels fibronectin and alters cancer cell migration*. Sci Rep, 2015. **5**: p. 8334.
- 68. Fullar, A., et al., *Remodeling of extracellular matrix by normal and tumor-associated fibroblasts promotes cervical cancer progression.* BMC Cancer, 2015. **15**: p. 256.
- 69. Erdogan, B. and D.J. Webb, *Cancer-associated fibroblasts modulate growth factor signaling and extracellular matrix remodeling to regulate tumor metastasis.* Biochem Soc Trans, 2017. **45**(1): p. 229-236.
- 70. Glentis, A., et al., *Cancer-associated fibroblasts induce metalloprotease-independent cancer cell invasion of the basement membrane*. Nat Commun, 2017. **8**(1): p. 924.
- 71. Labernadie, A., et al., A mechanically active heterotypic E-cadherin/N-cadherin adhesion enables fibroblasts to drive cancer cell invasion. Nat Cell Biol, 2017. **19**(3): p. 224-237.
- 72. Broders-Bondon, F., et al., *Mechanotransduction in tumor progression: The dark side of the force.* J Cell Biol, 2018. **217**(5): p. 1571-1587.
- 73. Emon, B., et al., *Biophysics of Tumor Microenvironment and Cancer Metastasis A Mini Review*. Comput Struct Biotechnol J, 2018. **16**: p. 279-287.
- 74. Jang, I. and K.A. Beningo, *Integrins, CAFs and Mechanical Forces in the Progression of Cancer*. Cancers (Basel), 2019. **11**(5).
- 75. Nissen, N.I., M. Karsdal, and N. Willumsen, *Collagens and Cancer associated fibroblasts in the reactive stroma and its relation to Cancer biology.* J Exp Clin Cancer Res, 2019. **38**(1): p. 115.
- 76. Bertero, T., et al., *Tumor-Stroma Mechanics Coordinate Amino Acid Availability to Sustain Tumor Growth and Malignancy*. Cell Metab, 2019. **29**(1): p. 124-140 e10.
- 77. Elkabets, M., et al., *Human tumors instigate granulin-expressing hematopoietic cells that promote malignancy by activating stromal fibroblasts in mice.* J Clin Invest, 2011. **121**(2): p. 784-99.
- 78. Nielsen, S.R., et al., *Macrophage-secreted granulin supports pancreatic cancer metastasis by inducing liver fibrosis.* Nat Cell Biol, 2016. **18**(5): p. 549-60.
- 79. Strutz, F., et al., *Identification and characterization of a fibroblast marker: FSP1.* J Cell Biol, 1995. **130**(2): p. 393-405.
- Park, J.E., et al., Fibroblast activation protein, a dual specificity serine protease expressed in reactive human tumor stromal fibroblasts. J Biol Chem, 1999. 274(51): p. 36505-12.
- 81. Sugimoto, H., et al., *Identification of fibroblast heterogeneity in the tumor microenvironment*. Cancer Biol Ther, 2006. **5**(12): p. 1640-6.
- 82. Kelly, T., et al., *Fibroblast activation protein-alpha: a key modulator of the microenvironment in multiple pathologies.* Int Rev Cell Mol Biol, 2012. **297**: p. 83-116.

- 83. Ostman, A., *PDGF receptors in tumor stroma: Biological effects and associations with prognosis and response to treatment.* Adv Drug Deliv Rev, 2017. **121**: p. 117-123.
- 84. Primac, I., et al., *Stromal integrin alphal1 regulates PDGFR-beta signaling and promotes breast cancer progression.* J Clin Invest, 2019. **129**(11): p. 4609-4628.
- 85. Bonneau, C., et al., *A subset of activated fibroblasts is associated with distant relapse in early luminal breast cancer*. Breast Cancer Res, 2020. **22**(1): p. 76.
- 86. Li, H., et al., Reference component analysis of single-cell transcriptomes elucidates cellular heterogeneity in human colorectal tumors. Nat Genet, 2017. **49**(5): p. 708-718.
- 87. Bartoschek, M., et al., *Spatially and functionally distinct subclasses of breast cancerassociated fibroblasts revealed by single cell RNA sequencing.* Nat Commun, 2018. **9**(1): p. 5150.
- 88. Biffi, G., et al., *IL1-Induced JAK/STAT Signaling Is Antagonized by TGFbeta to Shape CAF Heterogeneity in Pancreatic Ductal Adenocarcinoma*. Cancer Discov, 2019. **9**(2): p. 282-301.
- 89. Elyada, E., et al., Cross-Species Single-Cell Analysis of Pancreatic Ductal Adenocarcinoma Reveals Antigen-Presenting Cancer-Associated Fibroblasts. Cancer Discov, 2019. **9**(8): p. 1102-1123.
- 90. Dominguez, C.X., et al., Single-Cell RNA Sequencing Reveals Stromal Evolution into LRRC15(+) Myofibroblasts as a Determinant of Patient Response to Cancer Immunotherapy. Cancer Discov, 2020. 10(2): p. 232-253.
- 91. Lambrechts, D., et al., *Phenotype molding of stromal cells in the lung tumor microenvironment*. Nat Med, 2018. **24**(8): p. 1277-1289.
- 92. Puram, S.V., et al., *Single-Cell Transcriptomic Analysis of Primary and Metastatic Tumor Ecosystems in Head and Neck Cancer*. Cell, 2017. **171**(7): p. 1611-1624 e24.
- 93. Harper, J. and R.C. Sainson, *Regulation of the anti-tumour immune response by cancer-associated fibroblasts.* Semin Cancer Biol, 2014. **25**: p. 69-77.
- 94. Poggi, A., et al., *Mechanisms of tumor escape from immune system: role of mesenchymal stromal cells.* Immunol Lett, 2014. **159**(1-2): p. 55-72.
- 95. Chen, X. and E. Song, *Turning foes to friends: targeting cancer-associated fibroblasts.* Nat Rev Drug Discov, 2019. **18**(2): p. 99-115.
- 96. Orimo, A., et al., Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell, 2005. **121**(3): p. 335-48.
- 97. Jung, D.W., et al., *Tumor-stromal crosstalk in invasion of oral squamous cell carcinoma: a pivotal role of CCL7.* Int J Cancer, 2010. **127**(2): p. 332-44.
- 98. O'Connell, J.T., et al., *VEGF-A and Tenascin-C produced by S100A4+ stromal cells are important for metastatic colonization.* Proc Natl Acad Sci U S A, 2011. **108**(38): p. 16002-7.
- 99. Calon, A., D.V. Tauriello, and E. Batlle, *TGF-beta in CAF-mediated tumor growth and metastasis*. Semin Cancer Biol, 2014. **25**: p. 15-22.
- Yu, Y., et al., Cancer-associated fibroblasts induce epithelial-mesenchymal transition of breast cancer cells through paracrine TGF-beta signalling. Br J Cancer, 2014. 110(3): p. 724-32.
- 101. Bai, Y.P., et al., *FGF-1/-3/FGFR4 signaling in cancer-associated fibroblasts* promotes tumor progression in colon cancer through Erk and MMP-7. Cancer Sci, 2015. **106**(10): p. 1278-87.

- 102. Zhuang, J., et al., *TGFbeta1 secreted by cancer-associated fibroblasts induces* epithelial-mesenchymal transition of bladder cancer cells through lncRNA-ZEB2NAT. Sci Rep, 2015. **5**: p. 11924.
- 103. Salmon, H., 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): p. 899-910.
- 104. Ene-Obong, A., et al., Activated pancreatic stellate cells sequester CD8+ T cells to reduce their infiltration of the juxtatumoral compartment of pancreatic ductal adenocarcinoma. Gastroenterology, 2013. **145**(5): p. 1121-32.
- 105. Attieh, Y., et al., *Cancer-associated fibroblasts lead tumor invasion through integrinbeta3-dependent fibronectin assembly.* J Cell Biol, 2017. **216**(11): p. 3509-3520.
- 106. Liu, T., et al., *Cancer-associated fibroblasts: an emerging target of anti-cancer immunotherapy*. J Hematol Oncol, 2019. **12**(1): p. 86.
- 107. Monteran, L. and N. Erez, *The Dark Side of Fibroblasts: Cancer-Associated Fibroblasts as Mediators of Immunosuppression in the Tumor Microenvironment.* Front Immunol, 2019. **10**: p. 1835.
- 108. Barrett, R. and E. Pure, *Cancer-associated fibroblasts: key determinants of tumor immunity and immunotherapy*. Curr Opin Immunol, 2020. **64**: p. 80-87.
- 109. Nazareth, M.R., et al., *Characterization of human lung tumor-associated fibroblasts and their ability to modulate the activation of tumor-associated T cells.* J Immunol, 2007. **178**(9): p. 5552-62.
- Pinchuk, I.V., et al., *PD-1 ligand expression by human colonic myofibroblasts/fibroblasts regulates CD4+ T-cell activity.* Gastroenterology, 2008. 135(4): p. 1228-1237, 1237 e1-2.
- 111. Lakins, M.A., et al., *Cancer-associated fibroblasts induce antigen-specific deletion of CD8* (+) *T Cells to protect tumour cells*. Nat Commun, 2018. **9**(1): p. 948.
- Gorchs, L., et al., Human Pancreatic Carcinoma-Associated Fibroblasts Promote Expression of Co-inhibitory Markers on CD4(+) and CD8(+) T-Cells. Front Immunol, 2019. 10: p. 847.
- 113. Khalili, J.S., et al., Oncogenic BRAF(V600E) promotes stromal cell-mediated immunosuppression via induction of interleukin-1 in melanoma. Clin Cancer Res, 2012. **18**(19): p. 5329-40.
- 114. Li, Z., et al., *Cancer-associated fibroblasts promote PD-L1 expression in mice cancer cells via secreting CXCL5.* Int J Cancer, 2019. **145**(7): p. 1946-1957.
- 115. Wang, J., et al., *Characterization of phosphoglycerate kinase-1 expression of stromal cells derived from tumor microenvironment in prostate cancer progression*. Cancer Res, 2010. **70**(2): p. 471-80.
- 116. Fiaschi, T., et al., *Reciprocal metabolic reprogramming through lactate shuttle coordinately influences tumor-stroma interplay.* Cancer Res, 2012. **72**(19): p. 5130-40.
- 117. Shan, T., et al., *Cancer-associated fibroblasts enhance pancreatic cancer cell invasion by remodeling the metabolic conversion mechanism.* Oncol Rep, 2017. **37**(4): p. 1971-1979.
- Cruz-Bermudez, A., et al., Cancer-associated fibroblasts modify lung cancer metabolism involving ROS and TGF-beta signaling. Free Radic Biol Med, 2019. 130: p. 163-173.
- 119. Sakamoto, A., et al., *Pyruvate secreted from patient-derived cancer-associated fibroblasts supports survival of primary lymphoma cells.* Cancer Sci, 2019. **110**(1): p. 269-278.

- 120. Becker, L.M., et al., *Epigenetic Reprogramming of Cancer-Associated Fibroblasts Deregulates Glucose Metabolism and Facilitates Progression of Breast Cancer.* Cell Rep, 2020. **31**(9): p. 107701.
- 121. Pavlides, S., et al., *The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma.* Cell Cycle, 2009. **8**(23): p. 3984-4001.
- 122. Sun, P., et al., *miR-186 regulates glycolysis through Glut1 during the formation of cancer-associated fibroblasts.* Asian Pac J Cancer Prev, 2014. **15**(10): p. 4245-50.
- 123. Comito, G., et al., Lactate modulates CD4(+) T-cell polarization and induces an immunosuppressive environment, which sustains prostate carcinoma progression via TLR8/miR21 axis. Oncogene, 2019. **38**(19): p. 3681-3695.
- 124. Fallarino, F., et al., *T cell apoptosis by tryptophan catabolism*. Cell Death Differ, 2002. **9**(10): p. 1069-77.
- 125. Meisel, R., et al., *Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation.* Blood, 2004. **103**(12): p. 4619-21.
- 126. Ino, Y., et al., Arginase II expressed in cancer-associated fibroblasts indicates tissue hypoxia and predicts poor outcome in patients with pancreatic cancer. PLoS One, 2013. **8**(2): p. e55146.
- 127. Timosenko, E., A.V. Hadjinicolaou, and V. Cerundolo, *Modulation of cancer-specific immune responses by amino acid degrading enzymes*. Immunotherapy, 2017. **9**(1): p. 83-97.
- 128. Chang, S.H., et al., Role of prostaglandin E2-dependent angiogenic switch in cyclooxygenase 2-induced breast cancer progression. Proc Natl Acad Sci U S A, 2004. 101(2): p. 591-6.
- 129. Pace, E., et al., *Role of prostaglandin E2 in the invasiveness, growth and protection of cancer cells in malignant pleuritis.* Eur J Cancer, 2006. **42**(14): p. 2382-9.
- 130. Li, P., et al., *Epigenetic silencing of microRNA-149 in cancer-associated fibroblasts mediates prostaglandin E2/interleukin-6 signaling in the tumor microenvironment.* Cell Res, 2015. **25**(5): p. 588-603.
- 131. Wang, D. and R.N. DuBois, *The Role of Prostaglandin E(2) in Tumor-Associated Immunosuppression*. Trends Mol Med, 2016. **22**(1): p. 1-3.
- 132. Prima, V., et al., *COX2/mPGES1/PGE2 pathway regulates PD-L1 expression in tumor-associated macrophages and myeloid-derived suppressor cells.* Proc Natl Acad Sci U S A, 2017. **114**(5): p. 1117-1122.
- 133. Kock, A., et al., Inhibition of Microsomal Prostaglandin E Synthase-1 in Cancer-Associated Fibroblasts Suppresses Neuroblastoma Tumor Growth. EBioMedicine, 2018. **32**: p. 84-92.
- 134. Snijdewint, F.G., et al., *Prostaglandin E2 differentially modulates cytokine secretion profiles of human T helper lymphocytes.* J Immunol, 1993. **150**(12): p. 5321-9.
- Harris, S.G., et al., *Prostaglandins as modulators of immunity*. Trends Immunol, 2002.
  23(3): p. 144-50.
- 136. Baratelli, F., et al., *Prostaglandin E2 induces FOXP3 gene expression and T regulatory cell function in human CD4+ T cells.* J Immunol, 2005. **175**(3): p. 1483-90.
- Sharma, S., et al., Tumor cyclooxygenase-2/prostaglandin E2-dependent promotion of FOXP3 expression and CD4+ CD25+ T regulatory cell activities in lung cancer. Cancer Res, 2005. 65(12): p. 5211-20.
- 138. Miao, J., et al., Prostaglandin E2 and PD-1 mediated inhibition of antitumor CTL responses in the human tumor microenvironment. Oncotarget, 2017. **8**(52): p. 89802-89810.

- 139. Li, A., et al., *Histone deacetylase 6 regulates the immunosuppressive properties of cancer-associated fibroblasts in breast cancer through the STAT3-COX2-dependent pathway.* Oncogene, 2018. **37**(45): p. 5952-5966.
- 140. Karavitis, J., et al., *Regulation of COX2 expression in mouse mammary tumor cells controls bone metastasis and PGE2-induction of regulatory T cell migration.* PLoS One, 2012. **7**(9): p. e46342.
- 141. Tong, D., et al., *The roles of the COX2/PGE2/EP axis in therapeutic resistance*. Cancer Metastasis Rev, 2018. **37**(2-3): p. 355-368.
- 142. Zhu, Y., et al., *High COX-2 expression in cancer-associated fibiroblasts contributes to poor survival and promotes migration and invasiveness in nasopharyngeal carcinoma*. Mol Carcinog, 2020. **59**(3): p. 265-280.
- 143. He, X.J., et al., *Expression of galectin-1 in carcinoma-associated fibroblasts promotes gastric cancer cell invasion through upregulation of integrin beta1*. Cancer Sci, 2014. 105(11): p. 1402-10.
- 144. Tang, D., et al., *Cancer-associated fibroblasts promote angiogenesis in gastric cancer through galectin-1 expression*. Tumour Biol, 2016. **37**(2): p. 1889-99.
- 145. Perillo, N.L., et al., *Apoptosis of T cells mediated by galectin-1*. Nature, 1995. **378**(6558): p. 736-9.
- 146. Stillman, B.N., et al., *Galectin-3 and galectin-1 bind distinct cell surface glycoprotein receptors to induce T cell death.* J Immunol, 2006. **176**(2): p. 778-89.
- 147. Rabinovich, G.A. and M.A. Toscano, *Turning 'sweet' on immunity: galectin-glycan interactions in immune tolerance and inflammation*. Nat Rev Immunol, 2009. **9**(5): p. 338-52.
- 148. de Lourdes Mora-Garcia, M., et al., *Mesenchymal stromal cells derived from cervical cancer produce high amounts of adenosine to suppress cytotoxic T lymphocyte functions*. J Transl Med, 2016. **14**(1): p. 302.
- 149. Li, M.O., et al., *Transforming growth factor-beta regulation of immune responses*. Annu Rev Immunol, 2006. **24**: p. 99-146.
- 150. Sanjabi, S., M.M. Mosaheb, and R.A. Flavell, *Opposing effects of TGF-beta and IL-15 cytokines control the number of short-lived effector CD8+ T cells.* Immunity, 2009. 31(1): p. 131-44.
- 151. Tinoco, R., et al., *Cell-intrinsic transforming growth factor-beta signaling mediates virus-specific CD8+ T cell deletion and viral persistence in vivo.* Immunity, 2009. **31**(1): p. 145-57.
- 152. Kinoshita, T., et al., Forkhead box P3 regulatory T cells coexisting with cancer associated fibroblasts are correlated with a poor outcome in lung adenocarcinoma. Cancer Sci, 2013. **104**(4): p. 409-15.
- 153. Ouyang, W., et al., *TGF-beta cytokine signaling promotes CD8+ T cell development* and low-affinity CD4+ T cell homeostasis by regulation of interleukin-7 receptor alpha expression. Immunity, 2013. **39**(2): p. 335-46.
- 154. Travis, M.A. and D. Sheppard, *TGF-beta activation and function in immunity*. Annu Rev Immunol, 2014. **32**: p. 51-82.
- 155. Sanjabi, S., S.A. Oh, and M.O. Li, *Regulation of the Immune Response by TGF-beta: From Conception to Autoimmunity and Infection.* Cold Spring Harb Perspect Biol, 2017. **9**(6).
- 156. Batlle, E. and J. Massague, *Transforming Growth Factor-beta Signaling in Immunity and Cancer*. Immunity, 2019. **50**(4): p. 924-940.
- 157. Ahmadzadeh, M. and S.A. Rosenberg, *TGF-beta 1 attenuates the acquisition and expression of effector function by tumor antigen-specific human memory CD8 T cells.* J Immunol, 2005. **174**(9): p. 5215-23.

- 158. Thomas, D.A. and J. Massague, *TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance*. Cancer Cell, 2005. **8**(5): p. 369-80.
- 159. Stavnezer, J. and J. Kang, *The Surprising Discovery That TGFβ Specifically Induces the IgA Class Switch*. The Journal of Immunology, 2009. **182**(1): p. 5-7.
- 160. Shalapour, S., et al., *Inflammation-induced IgA+ cells dismantle anti-liver cancer immunity*. Nature, 2017. **551**(7680): p. 340-345.
- 161. Kato, T., et al., Cancer-Associated Fibroblasts Affect Intratumoral CD8(+) and FoxP3(+) T Cells Via IL6 in the Tumor Microenvironment. Clin Cancer Res, 2018.
   24(19): p. 4820-4833.
- 162. Tauriello, D.V.F., et al., *TGFbeta drives immune evasion in genetically reconstituted colon cancer metastasis.* Nature, 2018. **554**(7693): p. 538-543.
- 163. Vanpouille-Box, C., et al., *TGFbeta Is a Master Regulator of Radiation Therapy-Induced Antitumor Immunity*. Cancer Res, 2015. **75**(11): p. 2232-42.
- Lan, Y., et al., Enhanced preclinical antitumor activity of M7824, a bifunctional fusion protein simultaneously targeting PD-L1 and TGF-beta. Sci Transl Med, 2018. 10(424).
- 165. Sow, H.S., et al., *Combined Inhibition of TGF-beta Signaling and the PD-L1 Immune Checkpoint Is Differentially Effective in Tumor Models*. Cells, 2019. **8**(4).
- 166. Lind, H., et al., Dual targeting of TGF-beta and PD-L1 via a bifunctional anti-PD-L1/TGF-betaRII agent: status of preclinical and clinical advances. J Immunother Cancer, 2020. **8**(1).
- 167. Liao, D., et al., *Cancer associated fibroblasts promote tumor growth and metastasis by modulating the tumor immune microenvironment in a 4T1 murine breast cancer model.* PLoS One, 2009. **4**(11): p. e7965.
- 168. Kraman, M., et al., Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-alpha. Science, 2010. **330**(6005): p. 827-30.
- 169. Wang, L.C., et al., *Targeting fibroblast activation protein in tumor stroma with chimeric antigen receptor T cells can inhibit tumor growth and augment host immunity without severe toxicity.* Cancer Immunol Res, 2014. **2**(2): p. 154-66.
- 170. Ohshio, Y., et al., Cancer-associated fibroblast-targeted strategy enhances antitumor immune responses in dendritic cell-based vaccine. Cancer Sci, 2015. 106(2): p. 134-42.
- 171. Wang, X.W., et al., Increased expression of chitinase 3-like 1 is a prognosis marker for non-small cell lung cancer correlated with tumor angiogenesis. Tumour Biol, 2015. **36**(2): p. 901-7.
- 172. Chen, I.X., et al., *Blocking CXCR4 alleviates desmoplasia, increases T-lymphocyte infiltration, and improves immunotherapy in metastatic breast cancer.* Proc Natl Acad Sci U S A, 2019. **116**(10): p. 4558-4566.
- 173. Gunderson, A.J., et al., *Blockade of fibroblast activation protein in combination with radiation treatment in murine models of pancreatic adenocarcinoma*. PLoS One, 2019. **14**(2): p. e0211117.
- 174. Ford, K., et al., *NOX4 Inhibition Potentiates Immunotherapy by Overcoming Cancer-Associated Fibroblast-Mediated CD8 T-cell Exclusion from Tumors.* Cancer Res, 2020. **80**(9): p. 1846-1860.
- 175. Cohen, N., et al., Fibroblasts drive an immunosuppressive and growth-promoting microenvironment in breast cancer via secretion of Chitinase 3-like 1. Oncogene, 2017. **36**(31): p. 4457-4468.
- 176. Libreros, S., R. Garcia-Areas, and V. Iragavarapu-Charyulu, *CHI3L1 plays a role in cancer through enhanced production of pro-inflammatory/pro-tumorigenic and angiogenic factors.* Immunol Res, 2013. **57**(1-3): p. 99-105.

- 177. Ngernyuang, N., et al., *Chitinase 3 like 1 is associated with tumor angiogenesis in cervical cancer.* Int J Biochem Cell Biol, 2014. **51**: p. 45-52.
- 178. Wang, J., et al., *Elevated Serum Concentration of Chitinase 3-Like 1 is an Independent Prognostic Biomarker for Poor Survival in Lung Cancer Patients.* Cell Physiol Biochem, 2016. **38**(2): p. 461-8.
- 179. Geng, B., et al., *Chitinase 3-like 1-CD44 interaction promotes metastasis and epithelial-to-mesenchymal transition through beta-catenin/Erk/Akt signaling in gastric cancer.* J Exp Clin Cancer Res, 2018. **37**(1): p. 208.
- 180. Kim, D.H. and J.M. Choi, *Chitinase 3-like-1, a novel regulator of Th1/CTL responses, as a therapeutic target for increasing anti-tumor immunity.* BMB Rep, 2018. **51**(5): p. 207-208.
- 181. Qiu, Q.C., et al., *CHI3L1 promotes tumor progression by activating TGF-beta signaling pathway in hepatocellular carcinoma.* Sci Rep, 2018. **8**(1): p. 15029.
- 182. Yeo, I.J., et al., *Roles of chitinase 3-like 1 in the development of cancer, neurodegenerative diseases, and inflammatory diseases.* Pharmacol Ther, 2019. **203**: p. 107394.
- 183. Chiarugi, P., *Cancer-associated fibroblasts and macrophages: Friendly conspirators for malignancy*. Oncoimmunology, 2013. **2**(9): p. e25563.
- 184. Mantovani, A., et al., *Tumour-associated macrophages as treatment targets in oncology*. Nat Rev Clin Oncol, 2017. **14**(7): p. 399-416.
- 185. Komohara, Y. and M. Takeya, *CAFs and TAMs: maestros of the tumour microenvironment*. J Pathol, 2017. **241**(3): p. 313-315.
- 186. Comito, G., et al., *Cancer-associated fibroblasts and M2-polarized macrophages* synergize during prostate carcinoma progression. Oncogene, 2014. **33**(19): p. 2423-31.
- 187. Laoui, D., et al., Functional Relationship between Tumor-Associated Macrophages and Macrophage Colony-Stimulating Factor as Contributors to Cancer Progression. Front Immunol, 2014. 5: p. 489.
- 188. Kuen, J., et al., Pancreatic cancer cell/fibroblast co-culture induces M2 like macrophages that influence therapeutic response in a 3D model. PLoS One, 2017. 12(7): p. e0182039.
- 189. Miyake, M., et al., *CXCL1-Mediated Interaction of Cancer Cells with Tumor-Associated Macrophages and Cancer-Associated Fibroblasts Promotes Tumor Progression in Human Bladder Cancer.* Neoplasia, 2016. **18**(10): p. 636-646.
- 190. Takahashi, H., et al., *Cancer-associated fibroblasts promote an immunosuppressive microenvironment through the induction and accumulation of protumoral macrophages.* Oncotarget, 2017. **8**(5): p. 8633-8647.
- 191. Zhang, A., et al., *Cancer-associated fibroblasts promote M2 polarization of macrophages in pancreatic ductal adenocarcinoma.* Cancer Med, 2017. **6**(2): p. 463-470.
- 192. Zhou, J., et al., Cancer-Associated Fibroblasts Correlate with Tumor-Associated Macrophages Infiltration and Lymphatic Metastasis in Triple Negative Breast Cancer Patients. J Cancer, 2018. 9(24): p. 4635-4641.
- 193. Gok Yavuz, B., et al., *Cancer associated fibroblasts sculpt tumour microenvironment* by recruiting monocytes and inducing immunosuppressive PD-1(+) TAMs. Sci Rep, 2019. **9**(1): p. 3172.
- 194. Zhang, R., et al., *Cancer-associated fibroblasts enhance tumor-associated macrophages enrichment and suppress NK cells function in colorectal cancer.* Cell Death Dis, 2019. **10**(4): p. 273.

- 195. Powell, D.R. and A. Huttenlocher, *Neutrophils in the Tumor Microenvironment*. Trends Immunol, 2016. **37**(1): p. 41-52.
- 196. Ocana, A., et al., *Neutrophils in cancer: prognostic role and therapeutic strategies.* Mol Cancer, 2017. **16**(1): p. 137.
- 197. Fridlender, Z.G., et al., *Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN.* Cancer Cell, 2009. **16**(3): p. 183-94.
- 198. Cheng, Y., et al., *Cancer-associated fibroblasts induce PDL1+ neutrophils through the IL6-STAT3 pathway that foster immune suppression in hepatocellular carcinoma*. Cell Death Dis, 2018. **9**(4): p. 422.
- 199. Yang, L., S. Han, and Y. Sun, An IL6-STAT3 loop mediates resistance to PI3K inhibitors by inducing epithelial-mesenchymal transition and cancer stem cell expansion in human breast cancer cells. Biochem Biophys Res Commun, 2014. 453(3): p. 582-7.
- 200. Eichten, A., et al., *Resistance to Anti-VEGF Therapy Mediated by Autocrine IL6/STAT3 Signaling and Overcome by IL6 Blockade*. Cancer Res, 2016. **76**(8): p. 2327-39.
- 201. Zheng, X., et al., *IL-6/STAT3 axis initiated CAFs via up-regulating TIMP-1 which was attenuated by acetylation of STAT3 induced by PCAF in HCC microenvironment.* Cell Signal, 2016. **28**(9): p. 1314-24.
- 202. Wu, X., et al., *IL-6 secreted by cancer-associated fibroblasts promotes epithelialmesenchymal transition and metastasis of gastric cancer via JAK2/STAT3 signaling pathway.* Oncotarget, 2017. **8**(13): p. 20741-20750.
- 203. Notarangelo, T., et al., *IL6/STAT3 axis mediates resistance to BRAF inhibitors in thyroid carcinoma cells.* Cancer Lett, 2018. **433**: p. 147-155.
- 204. Ham, I.H., et al., *Targeting interleukin-6 as a strategy to overcome stroma-induced resistance to chemotherapy in gastric cancer*. Mol Cancer, 2019. **18**(1): p. 68.
- 205. Flavell, R.A., et al., *The polarization of immune cells in the tumour environment by TGFbeta*. Nat Rev Immunol, 2010. **10**(8): p. 554-67.
- 206. Popov, A., et al., Indoleamine 2,3-dioxygenase-expressing dendritic cells form suppurative granulomas following Listeria monocytogenes infection. J Clin Invest, 2006. **116**(12): p. 3160-70.
- 207. Han, Y., et al., Human CD14+ CTLA-4+ regulatory dendritic cells suppress T-cell response by cytotoxic T-lymphocyte antigen-4-dependent IL-10 and indoleamine-2,3-dioxygenase production in hepatocellular carcinoma. Hepatology, 2014. **59**(2): p. 567-79.
- 208. Cheng, J.T., et al., *Hepatic carcinoma-associated fibroblasts induce IDO-producing regulatory dendritic cells through IL-6-mediated STAT3 activation*. Oncogenesis, 2016. **5**: p. e198.
- 209. De Monte, L., et al., *Intratumor T helper type 2 cell infiltrate correlates with cancerassociated fibroblast thymic stromal lymphopoietin production and reduced survival in pancreatic cancer.* J Exp Med, 2011. **208**(3): p. 469-78.
- 210. Ma, Y., et al., Dynamic mast cell-stromal cell interactions promote growth of pancreatic cancer. Cancer Res, 2013. **73**(13): p. 3927-37.
- 211. Yang, Z., et al., *Mast cells mobilize myeloid-derived suppressor cells and Treg cells in tumor microenvironment via IL-17 pathway in murine hepatocarcinoma model*. PLoS One, 2010. **5**(1): p. e8922.
- 212. Deng, Y., et al., *Hepatic carcinoma-associated fibroblasts enhance immune suppression by facilitating the generation of myeloid-derived suppressor cells.* Oncogene, 2017. **36**(8): p. 1090-1101.

- 213. Xiang, H., et al., Cancer-Associated Fibroblasts Promote Immunosuppression by Inducing ROS-Generating Monocytic MDSCs in Lung Squamous Cell Carcinoma. Cancer Immunol Res, 2020. 8(4): p. 436-450.
- 214. Vivier, E., What is natural in natural killer cells? Immunol Lett, 2006. 107(1): p. 1-7.
- 215. Castriconi, R., et al., *Transforming growth factor beta 1 inhibits expression of NKp30 and NKG2D receptors: consequences for the NK-mediated killing of dendritic cells.* Proc Natl Acad Sci U S A, 2003. **100**(7): p. 4120-5.
- 216. Park, Y.P., et al., Complex regulation of human NKG2D-DAP10 cell surface expression: opposing roles of the gammac cytokines and TGF-beta1. Blood, 2011. **118**(11): p. 3019-27.
- 217. Donatelli, S.S., et al., *TGF-beta-inducible microRNA-183 silences tumor-associated natural killer cells.* Proc Natl Acad Sci U S A, 2014. **111**(11): p. 4203-8.
- 218. Laouar, Y., et al., *Transforming growth factor-beta controls T helper type 1 cell development through regulation of natural killer cell interferon-gamma*. Nat Immunol, 2005. **6**(6): p. 600-7.
- 219. Trotta, R., et al., *TGF-beta utilizes SMAD3 to inhibit CD16-mediated IFN-gamma production and antibody-dependent cellular cytotoxicity in human NK cells.* J Immunol, 2008. **181**(6): p. 3784-92.
- 220. Balsamo, M., et al., *Melanoma-associated fibroblasts modulate NK cell phenotype and antitumor cytotoxicity.* Proc Natl Acad Sci U S A, 2009. **106**(49): p. 20847-52.
- 221. Li, T., et al., *Colorectal carcinoma-derived fibroblasts modulate natural killer cell phenotype and antitumor cytotoxicity.* Med Oncol, 2013. **30**(3): p. 663.
- 222. Ziani, L., et al., Melanoma-associated fibroblasts decrease tumor cell susceptibility to NK cell-mediated killing through matrix-metalloproteinases secretion. Oncotarget, 2017. **8**(12): p. 19780-19794.
- 223. Young, A., et al., A2AR Adenosine Signaling Suppresses Natural Killer Cell Maturation in the Tumor Microenvironment. Cancer Res, 2018. **78**(4): p. 1003-1016.
- 224. Inoue, T., et al., *Cancer-associated fibroblast suppresses killing activity of natural killer cells through downregulation of poliovirus receptor (PVR/CD155), a ligand of activating NK receptor.* Int J Oncol, 2016. **49**(4): p. 1297-304.
- 225. Chiossone, L., et al., *Natural killer cell immunotherapies against cancer: checkpoint inhibitors and more.* Semin Immunol, 2017. **31**: p. 55-63.
- 226. Andre, P., et al., *Anti-NKG2A mAb Is a Checkpoint Inhibitor that Promotes Antitumor Immunity by Unleashing Both T and NK Cells.* Cell, 2018. **175**(7): p. 1731-1743 e13.
- 227. Habif, G., et al., *Targeting natural killer cells in solid tumors*. Cell Mol Immunol, 2019. **16**(5): p. 415-422.
- 228. Pickup, M.W., J.K. Mouw, and V.M. Weaver, *The extracellular matrix modulates the hallmarks of cancer*. EMBO Rep, 2014. **15**(12): p. 1243-53.
- 229. Lu, P., V.M. Weaver, and Z. Werb, *The extracellular matrix: a dynamic niche in cancer progression.* J Cell Biol, 2012. **196**(4): p. 395-406.
- 230. Sorokin, L., *The impact of the extracellular matrix on inflammation*. Nat Rev Immunol, 2010. **10**(10): p. 712-23.
- 231. Hartmann, N., et al., *Prevailing role of contact guidance in intrastromal T-cell trapping in human pancreatic cancer*. Clin Cancer Res, 2014. **20**(13): p. 3422-33.
- 232. Chen, P.L., et al., Analysis of Immune Signatures in Longitudinal Tumor Samples Yields Insight into Biomarkers of Response and Mechanisms of Resistance to Immune Checkpoint Blockade. Cancer Discov, 2016. **6**(8): p. 827-37.
- 233. Jiang, H., et al., *Targeting focal adhesion kinase renders pancreatic cancers responsive to checkpoint immunotherapy.* Nat Med, 2016. **22**(8): p. 851-60.

- 234. Kobayashi, N., et al., *Hyaluronan deficiency in tumor stroma impairs macrophage trafficking and tumor neovascularization.* Cancer Res, 2010. **70**(18): p. 7073-83.
- 235. Acerbi, I., et al., *Human breast cancer invasion and aggression correlates with ECM stiffening and immune cell infiltration.* Integr Biol (Camb), 2015. 7(10): p. 1120-34.
- 236. Ecker, B.L., et al., *Age-Related Changes in HAPLN1 Increase Lymphatic Permeability and Affect Routes of Melanoma Metastasis.* Cancer Discov, 2019. **9**(1): p. 82-95.
- 237. Goehrig, D., et al., *Stromal protein betaig-h3 reprogrammes tumour microenvironment in pancreatic cancer*. Gut, 2019. **68**(4): p. 693-707.
- 238. Kaur, A., et al., Remodeling of the Collagen Matrix in Aging Skin Promotes Melanoma Metastasis and Affects Immune Cell Motility. Cancer Discov, 2019. 9(1): p. 64-81.
- 239. Pereira, B.A., et al., *CAF Subpopulations: A New Reservoir of Stromal Targets in Pancreatic Cancer*. Trends Cancer, 2019. **5**(11): p. 724-741.
- 240. Santos, A.M., et al., *Targeting fibroblast activation protein inhibits tumor stromagenesis and growth in mice.* J Clin Invest, 2009. **119**(12): p. 3613-25.
- 241. Loeffler, M., et al., *Targeting tumor-associated fibroblasts improves cancer chemotherapy by increasing intratumoral drug uptake*. J Clin Invest, 2006. **116**(7): p. 1955-62.
- 242. Duperret, E.K., et al., Alteration of the Tumor Stroma Using a Consensus DNA Vaccine Targeting Fibroblast Activation Protein (FAP) Synergizes with Antitumor Vaccine Therapy in Mice. Clin Cancer Res, 2018. **24**(5): p. 1190-1201.
- 243. Ostermann, E., et al., *Effective immunoconjugate therapy in cancer models targeting a serine protease of tumor fibroblasts*. Clin Cancer Res, 2008. **14**(14): p. 4584-92.
- 244. Fang, J., et al., A potent immunotoxin targeting fibroblast activation protein for treatment of breast cancer in mice. Int J Cancer, 2016. **138**(4): p. 1013-23.
- 245. Welt, S., et al., Antibody targeting in metastatic colon cancer: a phase I study of monoclonal antibody F19 against a cell-surface protein of reactive tumor stromal fibroblasts. J Clin Oncol, 1994. **12**(6): p. 1193-203.
- 246. Scott, A.M., et al., A Phase I dose-escalation study of sibrotuzumab in patients with advanced or metastatic fibroblast activation protein-positive cancer. Clin Cancer Res, 2003. **9**(5): p. 1639-47.
- 247. Hofheinz, R.D., et al., *Stromal antigen targeting by a humanised monoclonal antibody: an early phase II trial of sibrotuzumab in patients with metastatic colorectal cancer.* Onkologie, 2003. **26**(1): p. 44-8.
- 248. Lo, A., et al., *Tumor-Promoting Desmoplasia Is Disrupted by Depleting FAP-Expressing Stromal Cells.* Cancer Res, 2015. **75**(14): p. 2800-2810.
- 249. Roberts, E.W., 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): p. 1137-51.
- 250. Melero, I., et al., *Clinical activity, safety, and PK/PD from a phase I study of RO6874281, a fibroblast activation protein (FAP) targeted interleukin-2 variant (IL-2v).* Annals of Oncology, 2018. **29**: p. viii134-viii135.
- 251. Dasari, S., Y. Fang, and A.K. Mitra, *Cancer Associated Fibroblasts: Naughty Neighbors That Drive Ovarian Cancer Progression.* Cancers (Basel), 2018. **10**(11).
- 252. Wang, H.C., L.P. Chan, and S.F. Cho, *Targeting the Immune Microenvironment in the Treatment of Head and Neck Squamous Cell Carcinoma*. Front Oncol, 2019. **9**: p. 1084.
- 253. Horvath, L., et al., Overcoming immunotherapy resistance in non-small cell lung cancer (NSCLC) novel approaches and future outlook. Mol Cancer, 2020. **19**(1): p. 141.

- 254. Joshi, S., *Targeting the Tumor Microenvironment in Neuroblastoma: Recent Advances and Future Directions.* Cancers (Basel), 2020. **12**(8).
- 255. Claus, C., et al., *Tumor-targeted 4-1BB agonists for combination with T cell bispecific antibodies as off-the-shelf therapy*. Sci Transl Med, 2019. **11**(496).
- 256. Trub, M., et al., Fibroblast activation protein-targeted-4-1BB ligand agonist amplifies effector functions of intratumoral T cells in human cancer. J Immunother Cancer, 2020. **8**(2).
- 257. Murakami, M., et al., *Docetaxel conjugate nanoparticles that target alpha-smooth muscle actin-expressing stromal cells suppress breast cancer metastasis.* Cancer Res, 2013. **73**(15): p. 4862-71.
- 258. Froeling, F.E., et al., *Retinoic acid-induced pancreatic stellate cell quiescence reduces paracrine Wnt-beta-catenin signaling to slow tumor progression*. Gastroenterology, 2011. **141**(4): p. 1486-97, 1497 e1-14.
- 259. Carapuca, E.F., et al., Anti-stromal treatment together with chemotherapy targets multiple signalling pathways in pancreatic adenocarcinoma. J Pathol, 2016. 239(3): p. 286-96.
- 260. Sherman, M.H., et al., *Vitamin D receptor-mediated stromal reprogramming suppresses pancreatitis and enhances pancreatic cancer therapy.* Cell, 2014. **159**(1): p. 80-93.
- 261. Kabacaoglu, D., et al., *Immune Checkpoint Inhibition for Pancreatic Ductal Adenocarcinoma: Current Limitations and Future Options.* Front Immunol, 2018. **9**: p. 1878.
- 262. Johnson, D.E., R.A. O'Keefe, and J.R. Grandis, *Targeting the IL-6/JAK/STAT3* signalling axis in cancer. Nat Rev Clin Oncol, 2018. **15**(4): p. 234-248.
- 263. Patel, R.A., et al., *RKI-1447 is a potent inhibitor of the Rho-associated ROCK kinases with anti-invasive and antitumor activities in breast cancer*. Cancer Res, 2012. **72**(19): p. 5025-34.
- 264. Hong, D., et al., *AZD9150*, a next-generation antisense oligonucleotide inhibitor of *STAT3 with early evidence of clinical activity in lymphoma and lung cancer*. Sci Transl Med, 2015. **7**(314): p. 314ra185.
- 265. Duluc, C., et al., *Pharmacological targeting of the protein synthesis mTOR/4E-BP1 pathway in cancer-associated fibroblasts abrogates pancreatic tumour chemoresistance*. EMBO Mol Med, 2015. **7**(6): p. 735-53.
- 266. Moatassim-Billah, S., et al., Anti-metastatic potential of somatostatin analog SOM230: Indirect pharmacological targeting of pancreatic cancer-associated fibroblasts. Oncotarget, 2016. 7(27): p. 41584-41598.
- 267. Pietras, K., et al., *Functions of paracrine PDGF signaling in the proangiogenic tumor stroma revealed by pharmacological targeting.* PLoS Med, 2008. **5**(1): p. e19.
- 268. Pietras, K., et al., *Inhibition of PDGF receptor signaling in tumor stroma enhances antitumor effect of chemotherapy*. Cancer Res, 2002. **62**(19): p. 5476-84.
- 269. Lefort, S., et al., *CXCR4 inhibitors could benefit to HER2 but not to triple-negative breast cancer patients*. Oncogene, 2017. **36**(9): p. 1211-1222.
- 270. Korpal, M., et al., *Imaging transforming growth factor-beta signaling dynamics and therapeutic response in breast cancer bone metastasis*. Nat Med, 2009. 15(8): p. 960-6.
- 271. Herbertz, S., et al., *Clinical development of galunisertib (LY2157299 monohydrate), a small molecule inhibitor of transforming growth factor-beta signaling pathway.* Drug Des Devel Ther, 2015. **9**: p. 4479-99.
- 272. Wei, Y., et al., *Fibroblast-specific inhibition of TGF-betal signaling attenuates lung and tumor fibrosis.* J Clin Invest, 2017. **127**(10): p. 3675-3688.

- 273. Yao, Y., et al., Artemisinin derivatives inactivate cancer-associated fibroblasts through suppressing TGF-beta signaling in breast cancer. J Exp Clin Cancer Res, 2018. **37**(1): p. 282.
- 274. Colak, S. and P. Ten Dijke, *Targeting TGF-beta Signaling in Cancer*. Trends Cancer, 2017. **3**(1): p. 56-71.
- 275. Kovacs, R.J., et al., Cardiac Safety of TGF-beta Receptor I Kinase Inhibitor LY2157299 Monohydrate in Cancer Patients in a First-in-Human Dose Study. Cardiovasc Toxicol, 2015. **15**(4): p. 309-23.
- 276. Brandes, A.A., et al., A Phase II randomized study of galunisertib monotherapy or galunisertib plus lomustine compared with lomustine monotherapy in patients with recurrent glioblastoma. Neuro Oncol, 2016. **18**(8): p. 1146-56.
- 277. Kelley, R.K., et al., A Phase 2 Study of Galunisertib (TGF-betal Receptor Type I Inhibitor) and Sorafenib in Patients With Advanced Hepatocellular Carcinoma. Clin Transl Gastroenterol, 2019. **10**(7): p. e00056.
- 278. Zhang, Z., et al., *Hyaluronan synthase 2 expressed by cancer-associated fibroblasts promotes oral cancer invasion.* J Exp Clin Cancer Res, 2016. **35**(1): p. 181.
- 279. McCarthy, J.B., D. El-Ashry, and E.A. Turley, *Hyaluronan, Cancer-Associated Fibroblasts and the Tumor Microenvironment in Malignant Progression.* Front Cell Dev Biol, 2018. **6**: p. 48.
- 280. Chauhan, V.P., et al., Angiotensin inhibition enhances drug delivery and potentiates chemotherapy by decompressing tumour blood vessels. Nat Commun, 2013. 4: p. 2516.
- 281. Provenzano, P.P., et al., *Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma*. Cancer Cell, 2012. **21**(3): p. 418-29.
- 282. Jacobetz, M.A., et al., *Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer*. Gut, 2013. **62**(1): p. 112-20.
- 283. Hingorani, S.R., et al., *Phase Ib Study of PEGylated Recombinant Human Hyaluronidase and Gemcitabine in Patients with Advanced Pancreatic Cancer.* Clin Cancer Res, 2016. **22**(12): p. 2848-54.
- 284. Olive, K.P., et al., Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science, 2009. **324**(5933): p. 1457-61.
- 285. Athar, M., et al., *Sonic hedgehog signaling in Basal cell nevus syndrome*. Cancer Res, 2014. **74**(18): p. 4967-75.
- 286. Kim, D.J., et al., *Open-label, exploratory phase II trial of oral itraconazole for the treatment of basal cell carcinoma*. J Clin Oncol, 2014. **32**(8): p. 745-51.
- 287. Brown, P.D., *Matrix metalloproteinase inhibitors: a novel class of anticancer agents.* Adv Enzyme Regul, 1995. **35**: p. 293-301.
- 288. Konstantinopoulos, P.A., et al., *Matrix metalloproteinase inhibitors as anticancer agents*. Int J Biochem Cell Biol, 2008. **40**(6-7): p. 1156-68.
- 289. Zucker, S. and J. Cao, *Selective matrix metalloproteinase (MMP) inhibitors in cancer therapy: ready for prime time?* Cancer Biol Ther, 2009. **8**(24): p. 2371-3.
- Winer, A., S. Adams, and P. Mignatti, *Matrix Metalloproteinase Inhibitors in Cancer Therapy: Turning Past Failures Into Future Successes*. Mol Cancer Ther, 2018. 17(6): p. 1147-1155.
- 291. Chiappori, A.A., et al., A phase I pharmacokinetic and pharmacodynamic study of s-3304, a novel matrix metalloproteinase inhibitor, in patients with advanced and refractory solid tumors. Clin Cancer Res, 2007. **13**(7): p. 2091-9.
- 292. Vandenbroucke, R.E. and C. Libert, *Is there new hope for therapeutic matrix metalloproteinase inhibition?* Nat Rev Drug Discov, 2014. **13**(12): p. 904-27.



immunotherapy



