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Review

Fibroblasts in cancer: Defining target structures for therapeutic intervention

Maximilian Boesch[a,*], Florent Baty[a], Holger Rumpold[b], Sieghart Sopper[c,e], Dominik Wolf[c,d], Martin H. Brutsche[a]

[a] Lung Center, Cantonal Hospital St. Gallen, Rorschacherstrasse 95, 9007 St. Gallen, Switzerland
[b] Internal Medicine II (Medical Oncology, Hematology, Gastroenterology & Rheumatology), Academic Teaching Hospital Feldkirch, Carinagasse 47, 6807 Feldkirch, Austria
[c] Internal Medicine V (Hematology & Oncology), Medical University of Innsbruck, Anichstrasse 35, 6020 Innsbruck, Austria
[d] Medical Clinic 3, Oncology, Hematology, Immunoncology and Rheumatology, University Clinic Bonn (UKB), Sigmund-Freud-Strasse 25, 53127 Bonn, Germany
[e] Tyrolean Cancer Research Institute (TKFI), Innrain 66, 6020 Innsbruck, Austria

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ABSTRACT

The functional importance of the tumor stroma for cancer growth and progression is increasingly recognized, but has not resulted in notable therapeutic developments yet. Within the mesenchymal tumor microenvironment, cancer-associated fibroblasts take the center stage and fuel tumor progression in various ways including malignant cell potentiation, immune regulation and fibrosis. However, recent studies have demonstrated pronounced heterogeneity of the fibroblastic tumor stroma, which comprises a plethora of individual cell subsets with varying phenotypes and functions, some of which suppress malignant growth through immune engagement or crosstalk with the tumor vasculature. This article summarizes the various levels at which the fibroblastic tumor stroma may impact cancer progression and highlights potential target structures for future therapeutic intervention(s).

1. Fibroblasts in cancer: from neglect to center stage

Systemic therapies of cancer have long focused on conferring *direct* damage to the malignant cells. This was traditionally accomplished using anti-proliferative drugs, such as cytotoxic or cytostatic chemotherapeutics, with partially good success. A downside of chemotherapy is its significant, often treatment-limiting toxicity towards healthy tissues with a naturally fast turnover. For instance, hematopoietic system toxicity [1] or unacceptable gastrointestinal toxicity [2] often lead to chemotherapy being abandoned or interrupted, which marks a delicate setback in the race against time. Another limitation of chemotherapy is its documented failure to eradicate the apex population of the intratumoral hierarchy, i.e., cancer stem cells (CSCs), which persist and propagate the tumor in both primary and secondary sites [3–6].

The tumor microenvironment (TME) comprises the totality of cellular and extracellular components that are present within, or immediately adjacent to, a tumor mass [7–9]. While historically, a 'bystander role' of the TME was assumed, recent data have shown remarkable dynamics within the tumor ecosystem including functional importance of mesenchymal and endothelial cells. It is now well-established that non-transformed cells of the TME underlie complex cellular diversification processes, and that they significantly – yet differentially – contribute to tumor progression. In recent years, sophisticated genetic tools [10–12] have allowed the dissection of the stromal infrastructure of tumors in unprecedented detail, which has revealed potential therapeutic leads. In the plethora of tumor microenvironmental cell types, cancer-associated fibroblasts (CAFs) have evolved as one of the most promising targets owing to their abundant presence and functional significance in various tumor entities. CAFs can be phenotypically identified based on markers such as FAP, αSMA and FSP-1, and arise from bone marrow-derived precursors and/or tissue-resident stromal cells through cancer cell-induced reprogramming [9,13]. CAF reprogramming/activation factors include, but are not limited to, TGF-β and other growth factors [13], the transcriptional regulator HSF1 [14], the inflammatory cytokine IL-1α [15], and reactive oxygen species resulting from chronic oxidative stress [16]. Based on paracrine and juxtacrine signals, these stromal cells modulate cancer progression both directly (malignant cell interaction) and indirectly (immune cell regulation), in which the net outcome depends on context as well as the

Abbreviations: CAF, Cancer-associated fibroblast; CSC, Cancer stem cell; ECM, Extracellular matrix; ELS, Ectopic lymphoid structure; EMT, Epithelial-to-mesenchymal transition; FRC, Fibroblastic reticular cell; FSC, Fibroblastic stromal cell; MSC, Mesenchymal stem/stromal cell; SLO, Secondary lymphoid organ

* Corresponding author at: Lung Center, Cantonal Hospital St. Gallen, Rorschacherstrasse 95, 9007 St. Gallen, Switzerland.
E-mail address: Maximilian.Boesch@kssg.ch (M. Boesch).

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particular phenotypic identity of a given population [13,17]. It is important to note that therapeutic strategies that employ fibroblasts hold several conceptual advantages that make them particularly appealing: (i) broad applicability owing to conserved patterns of the stromal architecture in different tumor types, (ii) less treatment adaptation/escape due to the genomic integrity of the target cells, and (iii) 'therapeutic flexibility' considering the existence of both tumor-propagating and –suppressive subsets [18–21]. Thus, CAFs have gained momentum in therapeutic anticancer discovery, and strategies that target these non-malignant cells for *indirect* cancer treatment are being actively pursued [22,23]. The hope is that CAF-targeted treatments can complement the already established immunotherapeutic approaches such as checkpoint blockade, and/or synergize with these in rational combination therapies [24].

This article summarizes the current knowledge about fibroblasts in cancer and reviews potential target structures for therapeutic intervention. The goal is to leverage the clinical development of fibroblast-based medicines in cancer.

2. Multilayered promotion of tumor growth

Fibroblasts originate from the mesodermal lineage and are thought to descend from a multipotent mesenchymal stem/stromal cell (MSC) [25]. Although encounter with malignant cells triggers CAF genesis from MSCs [26], the signals and pathways that orchestrate this differentiation process remain ill-defined. In addition, CAFs share many phenotypic and functional properties with MSCs including high expression of MSC-defining surface markers and pro-tumorigenic activity [27,28]. It is therefore likely that CAFs retain some principal characteristics of their primitive MSC precursors while adopting additional, more specialized, functions in the evolving TME.

One of the most obvious contributions of CAFs to tumor progression is their high-level production of growth factors including EGF, FGF2, PDGF-α/β, VEGF-A/C, IGF-I, HGF and CTGF, as well as a set of cytokines and chemokines including IL-6, IL-17A and CCL5 [13,17,23,29]. These secretory factors stimulate the proliferation (and may inhibit the apoptosis) of nearby cells that express the cognate receptor(s), such as cancer cells and vascular- and lymphatic endothelial cells. The net result is that efficient tumor growth can occur, potentially accompanied by hematogenous and/or lymphatic spread. A secondary effect is metabolic reprogramming within the TME [13,23,30], which may promote tumor progression through acidosis and/or hypoxia. Metabolic reprogramming is partially attributable to the master regulator NNMT, an epigenetic enzyme, which depletes S-adenosyl methionine and causes widespread proteomic changes in the tumor stroma that functionally converge in a tumor-promoting phenotype [31]. CAFs are also known to secrete cytokines that prompt glycogen breakdown and aerobic glycolysis in cancer cells, thus causing a funneled metabolic shift that ultimately facilitates tumor growth and metastasis [32,33]. However, metabolic signaling between tumor cells and CAFs occurs bi-directionally, with tumor cell-derived metabolites reciprocally affecting the behavior of CAFs, e.g., by imposing a regenerative response that largely resembles wound healing processes [34].

The production and deposition of excessive connective tissue material, commonly known as fibrosis, can also foster tumor progression, even though the underlying mechanisms remain to be fully elucidated [35]. It is believed that the various ECM components can stimulate the proliferation of cancer cells and that the formed scaffold is important for the malignant cells to establish footholds in the three-dimensional space, hence counteracting anoikis and supporting sphere-like growth [36–38]. In addition, progressive fibrosis causes mechanical stress and tissue stiffening [39], both of which may impair the vascular network to reduce perfusion and drug accessibility (drug resistance through 'physical exclusion'). In line, CAFs limit the intratumoral uptake of chemotherapeutic drugs including doxorubicin and 5-fluorouracil [40]. CAFs are also known to actively remodel the ECM through metalloproteases [41] as well as a DKK3-driven YAP/TAZ-dependent transcriptional program that enables actomyosin contractility thus leading to matrix stiffening [42]. Moreover, CAFs participate in the breaching of the basement membrane through a protease-independent mechanism [43]. CAFs may therefore pave the way for dissemination and metastasis, thus facilitating loco-regional as well as systemic involvement.

CAFs are major producers of epithelial-to-mesenchymal transition (EMT)-inducing factors, including TGF-β [44,45] and members of the Wnt family [46]. Moreover, a combined single-cell RNA/protein analytical pipeline recently showed that CAFs shape the pancreatic cancer architecture, with significant phenotypic shifts and enrichment of cancer cell populations with features of EMT and invasion [47]. Interestingly, these signatures are associated with particular glandular patterns as well as outcome, suggesting that clinical and morphological tumor characteristics can be traced back to particular molecular functions of CAFs during cancer development and progression. CAF-triggered adoption of mesenchymal features by carcinoma cells promotes tumor progression via two principal pathways: (i) treatment escape through de novo resistance [48] and (ii) acquisition of migratory potential, leading to metastasis [44–46]. On top of that, EMT of carcinoma cells is also associated with increased tumor cell stemness and tumor-initiating potential [49–51]. CAFs can also promote dissemination and metastasis through engaging in heterotypic interactions with tumor cells in so-called metastatic units, a mechanism that is thought to be especially relevant for cancers with propensity for peritoneal involvement and ascites formation (e.g., ovarian cancer) [52].

CAFs have been shown to tame the antitumor immune response through interfering with principal pathways required for optimal reactivity (immune modulation) [9,13] as well as excluding protective immune cell subsets from the tumor parenchyma [24]. CAFs have also been shown to curtail anticancer immunity by increasing the recruitment, differentiation and survival of $CD25^+FOXP3^+$ regulatory T cells [53,54]. CAF-derived PGE2 and IDO suppress the activation of NK cells, thus leading to reduced cytotoxicity and cytokine production [55,56], and may have regulating function also on the T cell response [57–59]. Similarly, TSLP expressed from CAFs polarizes a Th_2-response, which correlates with poor survival of pancreatic cancer patients [60]. CAFs have also been shown to neutralize the protective effects of IFN-γ and TNF-α during ongoing anticancer immune responses [11], and to provoke resistance to anti-PD-1 treatment through MMP-9 [61]. CAFs essentially contribute to tumor-promoting inflammation, and mediators of this effect include the interleukins IL-1β and IL-6, the chemokines CCL2 and CXCL5, and the enzyme COX-2 [62,63]. Many of these signals converge at the pro-inflammatory NF-κB hub, and lead to the recruitment of myeloid cells with tumor-promoting activity, including monocytes, macrophages and myeloid-derived suppressor cells [9,17,62]. IL-1α is another important secretory factor of the TME that signals through NF-κB. Produced by the neoplastic pancreas, IL-1α induces autocrine LIF in pancreatic stellate cells, leading to JAK/STAT activation and stromal reprogramming to an inflammatory CAF state with high expression of IL-6 and CXCL1 [15].

Aside from modulating treatment response via cancer cell plasticity, drug accessibility and immune regulation, CAFs can also confer direct therapy resistance to cancer cells. As an example, CAFs can provoke endocrine resistance in breast cancer cells [64] and may induce the upregulation of ABC drug transporters [65] extruding an array of chemotherapeutic and targeted drugs [66,67].

Collectively, CAFs contribute to tumor progression and drug resistance in various different ways (Fig. 1), explaining their association with poor survival of cancer patients [68–71].

3. Heterogeneity of the fibroblastic tumor stroma

It is well-established that fibroblasts, even in physiology and under homeostatic conditions, represent a heterogeneous cell pool, with

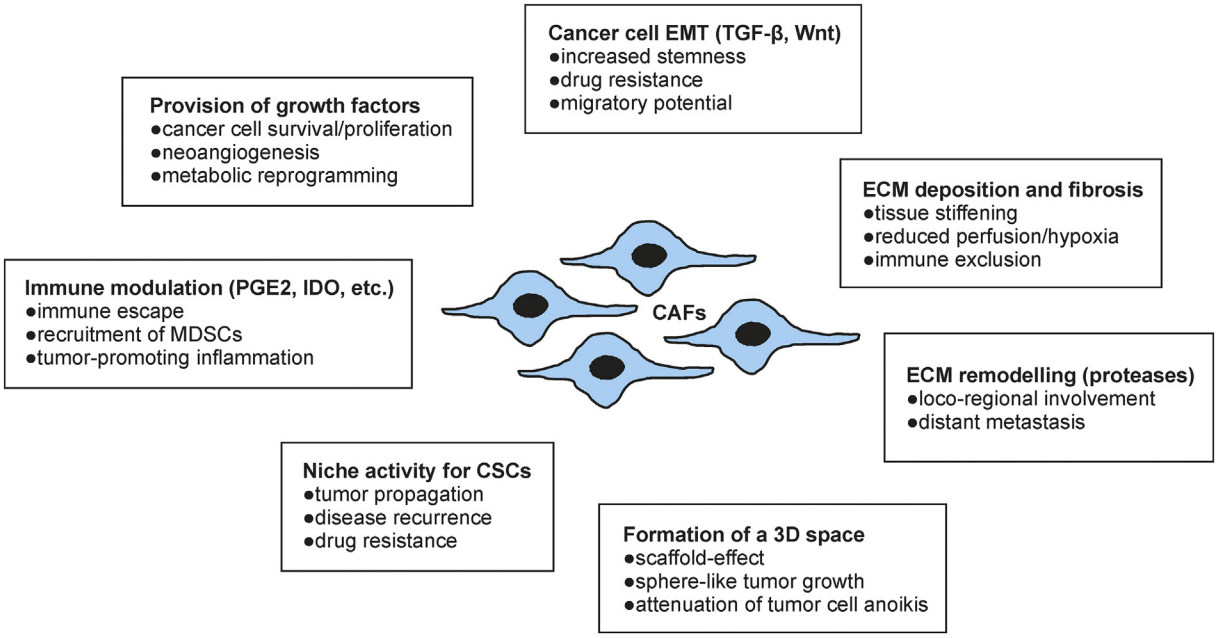

Fig. 1. Multilayered Roles of Cancer-Associated Fibroblasts in Tumor Progression. CAFs are implicated in various aspects of malignant growth, promoting cancer progression both directly (cancer cell interaction) and indirectly (e.g., immune regulation and neoangiogenesis). This renders CAFs and CAF-derived factors an attractive target for therapeutic intervention at different levels and stages including tumor initiation, metastatic progression, and remission after primary cytoreductive treatment. Abbreviations used: CAF, cancer-associated fibroblast; CSC, cancer stem cell; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; MDSC, myeloid-derived suppressor cell.

distinct and characteristic gene expression signatures to be found in different tissues and anatomical localizations [72–74]. In addition, skin developmental processes and wound repair are governed by different fibroblasts lineages that show distinct phenotypes and functions. As an example, lower dermis fibroblasts exhibiting Dlk1 expression provide the first wave of dermal regenerative capacity by laying down collagenous ECM and inducing fibrosis, while upper dermis fibroblasts expressing Blimp1 (and CD26) only populate the wound bed at later stages, selectively contributing to the papillary dermis during re-epithelialization [75]. Evidence suggests that the diversity, functional specialization and positional memory of fibroblasts is imprinted at least in part by transcription factors of the HOX gene family [72].

Considering this inherent variability of fibroblasts, it may not be surprising that the fibroblastic tumor stroma is plastic and heterogeneous as well, and that a plethora of phenotypically distinct CAF populations exist which differ in morphology, intratumoral localization and function [9,13,17]. The 'classical' pro-tumorigenic CAFs may reside more in the capsule region confining the tumor mass as well as in ECM-rich areas of the tumor parenchyma proper [19,76]. Other populations of 'CAFs', better termed fibroblastic stromal cells (FSCs) to clearly demarcate their converse functional behavior, are tumor-suppressive or neutral and may locate to particular niches in the tumor bed that foster the interaction with non-malignant cells, including immune- and endothelial cells [23,76–78]. Accordingly, these cells are morphologically distinct from classical CAFs and may resemble more the fibroblastic reticular cells (FRCs) of lymph nodes [12] or pericytes, the mural cells of blood vessels [79]. It is believed that different populations of tumor fibroblasts with various combinations of CAF- and FSC-specific features exist. According to this model, the fibroblastic TME represents the whole range of functional involvement, from clearly tumor-promoting to clearly tumor-suppressive, and neutral ('bystander' cells). The challenge is to delimit these opposing functions from each other and elucidate strategies for cell type-specific therapeutic intervention. This endeavor will also need to take into consideration adaptive and context-dependent functions of fibroblasts, including differential regulation in different microenvironments, and phenotypic and functional changes during tumor evolution as well as under the selective pressure of treatment.

Markers that have been used to define CAFs and FSCs include, but are not limited to, αSMA, FSP-1, FAP, PDPN, PDGFR-α/β, and NG2 [9,13]. However, most of these markers are also expressed in other cell compartments and hence lack specificity for CAFs/FSCs [9,13]. As an example, αSMA is a prototypical marker for vascular smooth muscle cells with contractile features [80], and PDPN is also expressed by lymphatic endothelial cells [81,82]. Similarly, NG2 and PDGFR-β are commonly used to identify pericytes [79]. It is important to note that CAF/FSC markers may underlie dynamic regulation and need not necessarily define homogeneous cell populations. In experimental breast cancer, co-expression of the hematopoietic growth factor IL-7 defined a minor – yet functionally relevant – subset of PDPN-expressing CAFs [19]. In the pancreatic cancer stroma, distinct populations of CAFs differentially contribute to desmoplasia and inflammation and are molecularly distinguishable through αSMA expression and IL-6 secretion [83]. Notably, an elegant study has revealed two discrete populations of FAP^+ stromal cells in immune cell-excluded tumors, a FAP^+PDPN^+ population with notable T cell-suppressing potential (CAFs) and a FAP^+PDPN^- population with neutral effects on the anticancer immune response (pericytes) [76]. CAF heterogeneity has also been reported in a recent article showing the differential accumulation and immune-modulating function of two myofibroblastic cell subsets, termed CAF-S1 and CAF-S4, in triple-negative breast cancer [53]. Moreover, immunohistochemical and functional analyses revealed a distinct subset of PDGFR-α^{low}/PDGFR-β^{high} CAFs driven and maintained by juxtacrine signaling between epithelial Jagged-1 and stromal NOTCH2 [84]. The presence of PDGFR-α^{low}/PDGFR-β^{high} CAFs in the peritumoral stroma identifies high-risk cases of ductal breast carcinoma in situ. Another study employed single-cell RNA sequencing of mesenchymal cells from a genetically-engineered mouse model of breast cancer to delineate three different populations of CAFs – mCAFs (matrix), vCAFs (vascular) and cCAFs (cycling) [85]. The genetic profiles of these populations carry independent prognostic significance in clinical breast cancer.

Taken together, the fibroblastic TME represents a phenotypically and functionally diverse component of the tumor stroma that influences tumor progression through various mechanisms. The second part of this review article deals with two particular functions of stromal cells in cancer, one tumor-promoting and one tumor-suppressive, in the quest of potential new targets for therapeutic intervention.

4. Fibroblasts bear niche activity for cancer stem cells

While the concept of CSCs as main drivers of cancer initiation, metastatic progression and recurrence after remission has sometimes faced skepticism in the past, the great therapeutic potential of stemness-depleting intervention is now definitely seen in the field [4,5,18,86]. Moreover, it is well-established that CSCs require a specialized niche for their survival and the maintenance of a primitive state [18,87–89]. Indeed, accumulating evidence suggests that CSCs not only engage in reciprocal interactions with their surrounding stroma but that they actively shape their microenvironment to secure sustained niche support ('architects of the tumor ecosystem') [6].

An early report established that POSTN expressed from αSMA^+VIM^+ stromal fibroblasts is required for the maintenance of $CD90^+CD24^+$ CSCs, and that targeting POSTN function counteracts metastasis [90]. Mechanistically, the authors showed that POSTN recruits particular Wnt family members through physical engagement, thereby inducing and amplifying Wnt signaling in the adjacent CSCs. Another study showed that STAT3-dependent production of CCL2 by CAFs stimulates a stem cell-specific program in breast cancer cells through activation of NOTCH signaling [91]. The CSC phenotype and drug resistance also seem to be coupled to CD44 expression on CAFs [92] and interestingly, targeting CD44 with an activating monoclonal antibody eradicates leukemic stem cells at least partially through a niche effect (interference with 'homing') [93]. An elegant study revealed a specific paracrine loop in which IGF-II expressed by $CD90^+$ CAFs induces Nanog expression in cancer cells, thereby fostering CSC enrichment through the process of dedifferentiation [94]. Of note, this stemness-related signaling circuit (more specifically, the combined signature $IGF\text{-}II^{hi}IGF1R^{hi}Nanog^{hi}$) is clinically relevant, predicting poor survival in stage I non-small cell lung cancer. Furthermore, a recent study disclosed a novel subset of CAFs defined by the two surface markers CD10 and GPR77 [95]. Clinically, these markers associate with disease progression and chemoresistance in unrelated tumor entities (i.e., breast and lung cancer). $CD10^+GPR77^+$ CAFs are molecularly characterized by persistent NF-κB activation and complement signaling, and facilitate tumor engraftment in patient-derived xenograft models.

In line with these observations, we recently demonstrated CSC niche activity of a particular subset of breast CAFs molecularly characterized by IL-7 expression [19]. Carrying a distinct transcriptomic signature with high expression of various stem cell-related factors, $IL\text{-}7^+$ CAFs preferentially located to marginal regions of the tumor known to harbor CXCR4-expressing CSCs [96]. Functionally, $IL\text{-}7^+$ CAFs were required for tumor propagation, and tumor-specific ablation of the cells slowed down tumor growth. Mechanistically, targeted interference with the CXCL12/CXCR4 axis through genetic or pharmacological approaches abolished tumor engraftment from a limiting cell dose, thus showing functional sustenance of tumor cell stemness by IL-7-expressing CAFs through CXCL12.

The aggregate of these data shows that diverse populations of CAFs support cancer stemness through various mechanisms and factors that concertedly form a dedicated niche for stem cell maintenance (Fig. 2). It follows that such subsets of CAFs represent a veritable therapeutic cancer target with widespread implications for the prevention of metastasis and relapse.

5. Immune stimulation and ectopic lymphoid structure formation

It is not an exaggeration to say that the last five years have witnessed a paradigm shift in medical oncology, with an ever-increasing use of immunotherapeutic and targeted approaches and a concurrent decline in cytotoxic/cytostatic modalities. Especially the impressive long-term responses observed with checkpoint inhibitors at least in a sub-fraction of patients [97] provide convincing evidence that a properly tuned immune system can really control and/or eradicate cancer *in the long run*. Complementing this therapeutic strategy of re-invigorating the endogenous immune response, cell-based treatment options such as chimeric antigen receptor (CAR)-T cell technology [98] provide protective anticancer immunity through re-infusion of ex vivo genetically-engineered and/or expanded immune effector cells; however, CAR-T cell therapies have so far yielded superb results only in hematological malignancies [99]. A third promising pillar in cancer immunotherapy is vaccination with tumor-specific antigens (i.e., neoantigens arising from cancer-specific mutations). This approach has been fully enabled only recently through the advent of next-generation sequencing platforms as well as the compelling advances in mutation calling and neoepitope prediction [100–102]. Vaccination with tumor-specific antigens is an inherently personalized approach and believed to provide unprecedented tumor selectivity thus diminishing the risks for autoimmunity [103]. Neoantigen vaccination may further synergize with checkpoint inhibitors and/or increase the response rates to checkpoint blockade [104].

A potential pitfall common to most of these approaches is the particular composition of the TME in immunologically 'cold' solid tumors [105]. In other words, the mounting of an efficient anticancer immune response is in vain if the tumor-reactive cells do not make their way into the tumor bed because of physical exclusion or if the systemic response is not properly supported in the tumor battlefield. Ectopic lymphoid structures (ELSs), or tertiary lymphoid organs, are organized lymphoid-like compartments that form preferentially at sites of chronic inflammation and cancer [106–108]. In these de novo arising structures, FSCs interact productively with immune cells and behave in many ways similar to their FRC counterparts in secondary lymphoid organs (SLOs) [107]. It is generally believed that ELSs inhibit cancer progression through granting immune cells access to tumor tissue and fostering tumor immune surveillance [109–113]; however, one report found evidence for accumulation of tumor-promoting regulatory T cells in ELSs [114]. FSCs in tumor ELSs are typically positive for the chemokines CCL21, CCL19 and CXCL13 [109,113,115] and support anticancer immunity through facilitating the interaction of T cells with antigen-presenting cells. Moreover, considering the importance of mesenchymal cells [116,117] and FRCs [12,118] for SLO development and function, specific subsets of FSCs may represent attractive targets for immune-potentiating treatment through the intentional induction of ELSs in tumor tissue. While therapeutic ELS induction may be further down the line, such strategies would conceptually try to mimic the molecular constellations during SLO development and/or harness the lymphocyte-retaining properties of specific immune-modulating drugs (see further details below).

FSCs can also engage in dynamic interactions with vascular endothelial cells, in which their definite differentiation from classical pericytes or myofibroblasts may not always be possible due to overlapping marker expression profiles and a similar localization and morphology [79]. In a recent publication, we disclosed pericyte-like features of lung tumor FSCs that expressed the homeostatic chemokine CCL19 [78], otherwise characteristic of FRCs in SLOs [12]. CCL19-producing FSCs expressed the pericyte marker NG2 [79] and spatially associated with CD31-expressing blood vessels which they also ensheathed. In clinical lung cancer samples and preclinical tumor models we found that the presence of a CCL19 signature correlated with preferential infiltration of the tumor with immune cells. Depletion of $CCL19^+$ FSCs from orthotopically growing lung tumors diminished T cell infiltration and unleashed tumor growth. These data suggested that CCL19-expressing FSCs grant immune cells access to the tumor bed, resulting in suppression of tumor growth. Importantly, we also

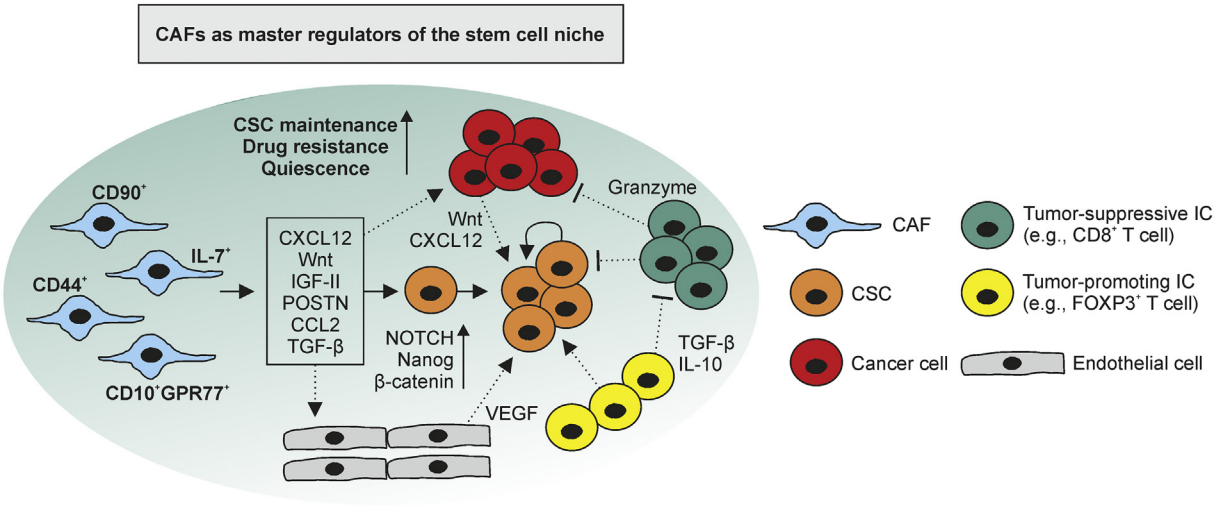

Fig. 2. Stem Cell Niche Activity of Cancer-Associated Fibroblasts. Particular populations of CAFs promote tumor stemness phenotypes by providing a specialized niche for CSCs. This niche is formed by the concerted action of various stem cell-related factors that jointly secure CSC maintenance and prevention of differentiation. Secondary effects are quiescence and drug resistance, which leads to CSC persistence thus predisposing to relapse. It follows that targeted inhibition of niche-forming CAFs may enable stemness-depleting intervention, hence tackling two of the most important issues in medical oncology, drug resistance and recurrence. The proposed phenotype of niche-forming CAFs is indicated, and the potential functional relationship between various cell populations of the stem cell niche is shown (not complete). Abbreviations used: CAF, cancer-associated fibroblast; CSC, cancer stem cell; IC, immune cell.

observed co-localization of $CCL19^+$ FSCs and $CD8^+$ T cells in specific intratumoral niches. Thus, CCL19-producing FSCs may foster tumor immune surveillance based on two distinct mechanisms: (i) vessel stabilization leading to enhanced perfusion and accessibility of immune cells and (ii) niche-associated immune cell accommodation accounting for ELS-like activity (Fig. 3).

Collectively, these data show that despite an overriding tumor-promoting function of the fibroblastic stroma and CAFs, certain fibroblastic cell subsets exert tumor-suppressive effects through immune engagement and vessel normalization.

6. Prospects for therapeutic development

The existence of both tumor-promoting and –suppressive populations of fibroblasts enables flexibility in the overall therapeutic concept (inhibition versus induction). Moreover, the intended effects of CAF/FSC targeting can be more on the tumor side or more on the microenvironmental side, employing, for instance, immune cells or endothelial cells. Thus, CAF/FSC targeting can be realized at different levels of the underlying tumor biology.

Generally, one needs to distinguish between fibroblast-derived *molecules* and fibroblasts as *cells*. Molecules and factors produced by CAFs/FSCs are targetable with small compound inhibitors, antibodies, peptides, etc. Conversely, the elimination or induction of a whole cell subset poses more complex challenges and is likely further down the road. While in vivo cell ablation is quite easily achievable in research settings through genetic engineering [10,19,20], translation into the clinics is considerably hindered by technical and ethical constraints. Accordingly, cell-level approaches may need to harness natural vulnerabilities of CAFs including synthetically lethal molecular constellations. On the other hand, tumor-suppressive FSC subsets might be inducible exploiting knowledge about their plasticity and particular requirements during development and repair-associated repopulation. While the clinical programs of drugs targeting CAFs have been reviewed in an excellent article [119], the scope of this paper is to define potential fibroblast-related therapeutic targets based on evidence from more fundamental research. Thus, this paper tries to spotlight promising strategies of CAF/FSC targeting that warrant clinical investigation in the future.

First and foremost, CAFs are an attractive target for stemness-depleting intervention. Stemness-related CAF targets include, but are not limited to, CCL2 [91], CD44 [92,93], CXCL12 [19], HGF [120], IGF-II [94] and POSTN [90]. Realization of corresponding targeting strategies using antibodies or small molecule inhibitors may significantly improve cancer outcome by disrupting the 'beating heart of tumors' [121], thereby tackling drug resistance and recurrence [4,18]. CAFs may also be harnessed to therapeutically address the main cause of cancer-related death, systemic spread. Metastasis-related CAF targets comprise angiogenic growth factors (FGF-2, PDGF-BB, VEGF-A) [122–124] and the ECM glycoprotein tenascin-C [124]. The growth factors are targetable with blocking antibodies or receptor antagonists, whereas tenascin-C is drugable with aptamers [125], peptides [126] and nanoliposomes [127]. CAFs also contribute to metabolic reprogramming within the TME [13,17] and synthesize metabolites and enzymes that are functionally important to sustain tumor growth. Two such CAF-related metabolic targets are the amino acid aspartate [128] and the enzyme glutamine synthetase [129], both targetable with low molecular weight drugs. Other strategies of CAF targeting aim to exploit their well-defined roles in immune regulation (e.g., TGF-β) [24] and fibrosis (e.g., HGF and BET proteins) [120,130], or to interfere with their differentiation or fate (e.g., IL-1, vitamin D receptor) [15,131].

The therapeutic exploitation of tumor-suppressive stromal elements requires strategies other than *inhibition* and may be more difficult to accomplish. A recurrent finding is that FSC populations with myofibroblast- and/or pericyte-like features, including αSMA expression and a distinct perivascular localization, inhibit tumor growth through various mechanisms [20,21,78,123]. The therapeutic induction of such cells in the TME represents an elegant way of treating cancer but requires significant knowledge about their developmental requirements including the relevant instructive signals enabling their differentiation from multipotent precursors. SHH, a morphogen and ligand for the hedgehog pathway, positively regulates the stromal content of pancreatic tumors at least in part by increasing the abundance of αSMA-expressing myofibroblasts, and acts to restrain cancer progression [21]. It remains to be seen whether provision of this factor, or alternative activation of the hedgehog pathway, can impair tumor growth mediated through protective FSC accumulation in the tumor bed. Another study has shown that PDGF-BB induces pericyte-fibroblast transition, which fuels tumor growth and metastasis [123]. Targeting the PDGF-BB/PDGFR-β axis may therefore increase pericyte coverage in tumor

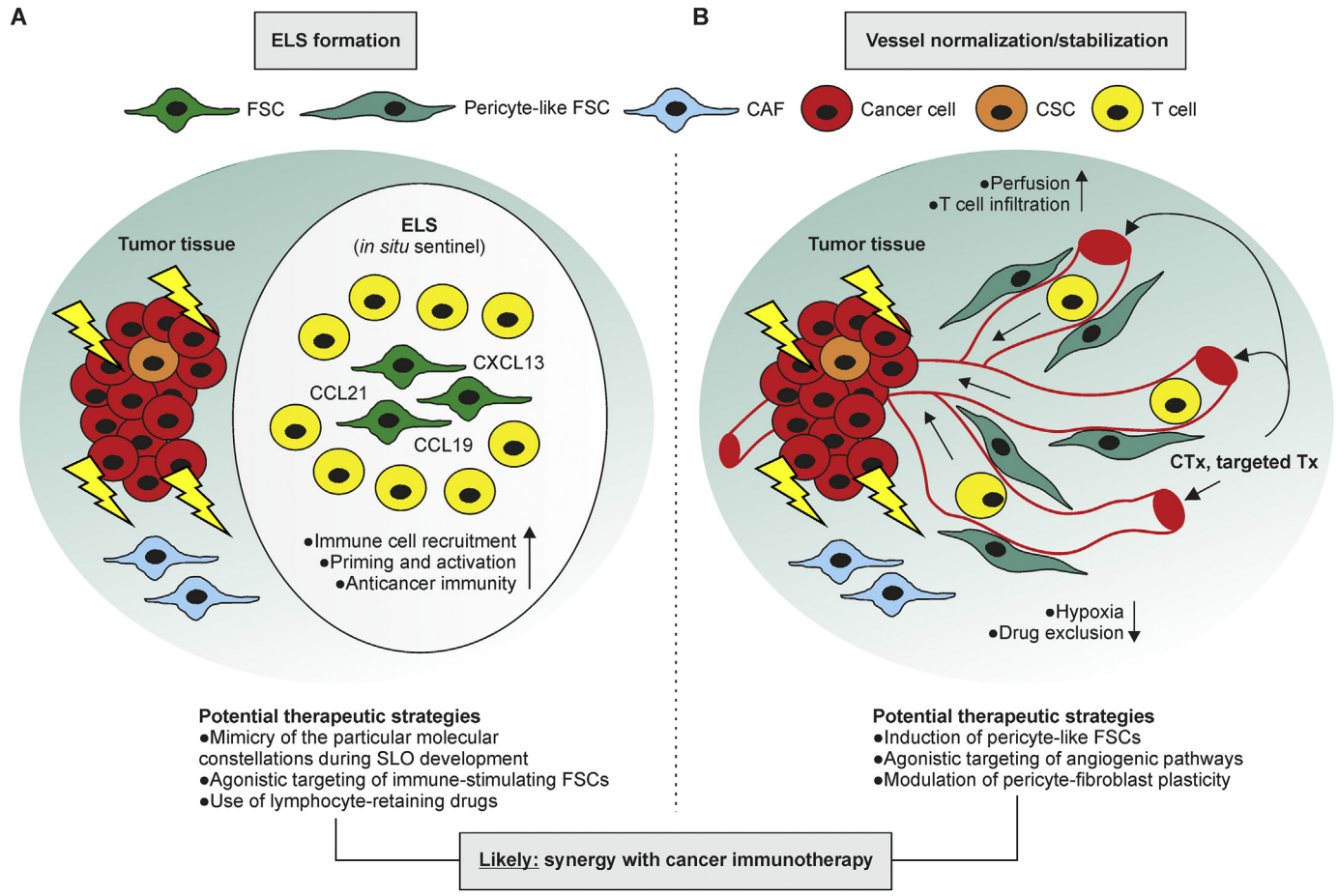

Fig. 3. Proposed Mechanisms of Immune Stimulation by Tumor Fibroblastic Stromal Cells. The tumor stroma harbors distinct populations of fibroblasts that vary in phenotype and function. Within this heterogeneity, certain subsets of FSCs bear tumor-suppressive activity by contributing to tumor immune surveillance in different ways. (A) ELS formation in tumor tissue. Particular subsets of FSCs produce immune cell-attracting chemokines and may resemble the mesenchymal lymphoid tissue organizer cells during SLO development. By this mechanism, FSCs may facilitate immune cell access to the tumor bed and provide a dedicated lymphoid niche for in situ antigen presentation and T cell activation. (B) Blood vessel normalization by tumor fibroblasts. Certain FSC subsets may associate with the tumor vasculature and exert pericyte-like functions to restore perfusion and blood flow through the tumor parenchyma. This may increase tumor immune cell infiltration and enhance drug accessibility for higher treatment efficacy, while reducing tumor-promoting hypoxia. (A + B) Potential therapeutic strategies are shown, and the likely synergy with (checkpoint-based) immunotherapy is indicated. Abbreviations used: CAF, cancer-associated fibroblast; CSC, cancer stem cell; CTx, chemotherapy; ELS, ectopic lymphoid structure; FSC, fibroblastic stromal cell; SLO, secondary lymphoid organ; targeted Tx, targeted therapy.

microvessels [123], thus stabilizing the otherwise chaotic tumor endothelium. Pericyte dropout from tumors may also be rescued by antibody-mediated Tie2 activation, which normalizes the tumor vasculature and produces a microenvironment permissive of immune cell infiltration and drug access [132]. A third strategy for pericyte-stimulating treatment is the intratumoral delivery of LIGHT, a member of the TNF superfamily, which restores vessel integrity, increases perfusion, and sensitizes to chemo- and immunotherapy [133].

The goal of therapeutically inducing ELSs specifically in the TME should emanate from the premise that tertiary lymphoid tissue formation follows similar developmental pathways as SLO (e.g., lymph node) organogenesis. Along this thought, specific molecular contributions from mesenchymal/fibroblastic cells [12,116,117] and vascular and lymphatic endothelial cells [134,135] are likely, and an obvious target is the lymphotoxin/LTβR axis. On the other hand, it has been shown that pharmacological inhibition of the sphingosine-1-phosphate receptor with the immunosuppressant fingolimod results in ectopic lymph node formation with a regular, underlying FRC scaffold at least in non-tumor settings [135]. While the use of immunosuppressive drugs is counterintuitive and questionable in cancer settings, the observation of compound-triggered de novo formation of lymphoid tissue is appealing and warrants consideration for the future development of ELS-inducing cancer therapies.

Conceptually, fibroblast-directed interventions would primarily target cytoreduced tumor settings including the various states of remission and minimal residual disease, with the intention to prevent later recurrence, a clinical objective that most current treatment modalities fall short of [18]. Conversely, fibroblast-targeted treatments, similarly as other molecularly targeted approaches, will be less suitable for conferring quick reductions in tumor load, and classical strategies such as debulking surgery and cytotoxic chemotherapy will remain the therapeutic mainstay here. Moreover, fibroblast-targeted treatments are predestined for rational combination therapies, and a good example here is the concomitant targeting of CAF-derived TGF-β (which enables T cell infiltration into otherwise immune-excluded tumors) and PD-L1-specific checkpoint inhibition (which 'releases the brake' to re-invigorate the otherwise restrained immune response) [24]. Furthermore, considering the chemo-sensitizing effects of CAF-targeted treatments [40], combinatorial approaches with classical cytotoxic/cytostatic drugs may also be envisaged.

It should also be noted that targeting tumor-promoting CAFs and/or immune-stimulating FSCs may produce on-target effects in the non-tumor-associated stroma, potentially resulting in systemic toxicity. While comprehensive clinical data on the side effect profile of fibroblast-targeting drugs are lacking, preclinical research has so far suggested a favorable safety profile of CAF-directed treatments [136].

Table 1
Potential targets for stroma-directed cancer therapy.

Tumor promotion → inhibition			
Target	Condition	Description	Ref.
Aspartate	Squamous carcinoma, breast cancer	Amino acid produced by CAFs that sustains cancer cell proliferation. Targetable with the SLC1A3 (aspartate transporter) blocker TFB-TBOA.	[128]
BET proteins	Pancreatic cancer	Chromatin reader proteins associated with desmoplastic stroma and tumor growth in patient-derived xenograft models. Linked to hedgehog/GLI1 and TGF-β signaling pathways. Targetable with JQ1.	[130]
CCL2	Breast cancer, lung cancer	C-C motif chemokine expressed in primary CAFs stimulating a stem cell-specific program in cancer cells including NOTCH1 induction. Important for reprogramming of normal fibroblasts into activated CAFs. Targetable with neutralizing antibodies.	[91,140]
CD44	Melanoma, lung cancer, AML	Cell surface glycoprotein supporting the stemness of cancer cells. Crucial for AML-LSC fate and niche homing. Targetable with antibodies and peptides.	[92,93,141]
CXCL12	Breast cancer, pancreatic cancer	C-X-C motif chemokine expressed from IL-7- and FAP-expressing CAFs. Niche activity for CSCs and impairing the immune response. Targetable with AMD3100 (receptor antagonist).	[19,142]
FGF-2	Colorectal cancer	Growth factor expressed by fibroblasts promoting contact-dependent migration of colorectal cancer cells. Targetable with blocking antibodies or small molecule inhibitors of the corresponding receptor (FGFR).	[122]
Glutamine synthetase	Ovarian cancer	Enzyme expressed in stromal cells/CAFs functionally important to sustain tumor growth. Synthetically lethal in combination with glutaminase inhibition. Targetable with methionine sulfoximine.	[129]
HGF	Hepatocellular carcinoma	Growth factor produced and secreted by CAFs. Enriches the fraction of liver tumor-initiating cells and may promote fibrosis. Targetable with neutralizing antibodies or the c-Met (receptor) inhibitor PHA-665752.	[120]
IGF-II	Lung cancer	Growth factor secreted by $CD90^+$ CAFs inducing Nanog expression and tumor cell stemness. Targetable with receptor (IGF1R)-specific blocking antibodies.	[94]
IL-1α	Pancreatic cancer	Cytokine produced by tumor cells stimulating an inflammatory phenotype in PSCs to generate 'inflammatory CAFs'. Process dependent on a LIF/JAK/STAT cascade and antagonized by TGF-β signaling. Targetable via multiple strategies, for instance with the JAK inhibitor AZD1480.	[15]
NOX4	HNSCC, colorectal cancer	NAD(P)H oxidase facilitating the accumulation of tumor-promoting, αSMA-expressing myofibroblasts. Targetable with GKT-831 (formerly GKT137831).	[143]
PDGF-BB	Squamous carcinoma	Growth factor inducing pericyte-fibroblast transition, leading to tumor growth and metastasis. Targetable with receptor (PDGFR-β)-specific TKIs such as imatinib.	[123]
POSTN	Breast cancer	Metastatic niche component recruiting Wnt ligands and securing CSC maintenance. Targetable with blocking antibodies.	[90]
Tenascin-C	Breast cancer, colorectal cancer	Glycoprotein of the ECM produced by $S100A4^+$ stromal cells (fibroblasts). Facilitates metastatic colonization and may counteract tumor cell apoptosis. Targetable with aptamers, peptides and nanoliposomes.	[124–127]
TGF-β	Metastatic urothelial carcinoma	Growth factor associated with fibroblasts and immune exclusion. Attenuates the response to checkpoint inhibition. Targetable with TGF-β-specific monoclonal antibodies such as 1D11.	[24]
VEGF-A	Breast cancer, colorectal cancer	Angiogenic growth factor expressed by $S100A4^+$ stromal cells (fibroblasts). Promotes metastatic colonization and modulates tumor angiogenesis. Targetable with neutralizing antibodies.	[124]
Vitamin D receptor	Pancreatic cancer	Transcriptional regulator of PSCs and expressed in the pancreatic tumor stroma. Allows stromal reprogramming for therapeutic benefit. Targetable with vitamin D analogs such as calcipotriol and paricalcitol. Currently tested in an early phase I trial (NCT03519308).	[131]
Tumor suppression → induction			
Target	Condition	Description	Ref.
αSMA*	Pancreatic cancer	Marker for a population of tumor fibroblasts whose presence associates with prolonged survival. Protective effects mediated through modulation of EMT, CSCs and anticancer immunity. Targeting/induction strategies to be elucidated.	[20]
CCL19*	Lung cancer	Homeostatic chemokine of SLOs and marker for an immune-stimulating population of tumor fibroblasts. Potentially linked to ELS formation. Targeting/induction strategies to be elucidated.	[78]
LIGHT	Breast cancer, pancreatic cancer	Inflammatory factor also known as TNFSF14. Facilitates pericyte differentiation and normalizes the tumor vasculature. Increases tumor perfusion and promotes the efficacy of chemo- and immunotherapy. Targeting/induction strategy: administration of recombinant protein.	[133]
SHH	Pancreatic cancer	Soluble ligand of the hedgehog signaling pathway. Positive regulation of the stromal tumor content and αSMA-expressing myofibroblasts. Targeting/induction strategies to be elucidated.	[21]
Other therapeutic anticancer strategies employing fibroblasts			
Strategy	Condition	Description	Ref.
Low-dose metronomic CTx	Breast cancer, pancreatic cancer	Shown to prevent stromal activation and phenotypic conversion of cancer cells into derivatives with tumor-initiating potential.	[144]

Note: This table does not claim completeness and specifies neither the level of supporting evidence nor the likelihood of successful clinical application. The purpose of this table is to provide an overview of potential stroma-related therapeutic targets/concepts for future drug development.
Abbreviations used: AML, acute myeloid leukemia; CAF, cancer-associated fibroblast; CSC, cancer stem cell; CTx, chemotherapy; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; HNSCC, head and neck squamous cell carcinoma; LSC, leukemic stem cell; PSC, pancreatic stellate cell; SLO, secondary lymphoid organ; TKI, tyrosine kinase inhibitor.

* Marker of a specific fibroblastic cell population (not necessarily a targetable molecule by itself).

Generally, the currently employed treatment modalities (including chemotherapy, targeted therapy and immunotherapy) are not devoid of side effects as well, and a certain amount of 'toxicity' may even be beneficial in terms of treatment response [137,138]. However, a systematic evaluation of the side effect profile of fibroblast-targeting drugs is certainly warranted, and further elucidation of cancer-specific fibroblast alterations (e.g., alterations induced by paracrine or juxtacrine tumor signals) will be instrumental to pave the way for more cancer cell-selective treatments.

Taken together, fibroblastic stroma-targeted treatments are versatile and able to tackle the malignant cascade at different levels and based on different, non-redundant mechanisms (Table 1). CAF and FSC targets,

including the ones highlighted here, therefore represent attractive leads for therapeutic development, possibly able to transform cancer medicine.

7. Concluding remarks

The progression of malignant disease is markedly shaped by CAFs and FSCs, suggesting these cells as rational therapeutic targets. However, compared to other modalities of indirect cancer treatment such as immunotherapy, the development of CAF/FSC-targeting treatments is lagging behind – for no good reason. Specifically, CAFs and FSCs offer many points of attack that are targetable with 'conventional' pharmaceuticals, such as antibodies and low molecular weight drugs. Moreover, CAF/FSC-directed therapies may be broadly applicable along with long lasting treatment efficacy, given the genomic stability of the targeted cells. An asset of CAF-directed treatment is the possibility of depriving the CSCs of their niche, which may counteract drug resistance and prevent recurrence [18,19]. On the other hand, the pool of CSCs has been recently shown to be causative for adaptive immune resistance following adoptive transfer of T cells [139]. Thus, the combination of CAF-based anti-CSC treatments with immunotherapy is an interesting therapeutic concept that warrants consideration for clinical development. Combining stroma-directed treatments with immunotherapy (e.g., checkpoint inhibition) also seems appealing in view of the established roles of stromal cells in immune modulation and vessel normalization [24,78,123,132].

Disclosure of potential conflicts of interest

There are no conflicts of interest to declare. There is also no non-author involvement in the preparation of the manuscript.

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