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**Fibroblast heterogeneity in solid cancer:
from single cell analysis to whole-body imaging**

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Abstract

Cancer-Associated Fibroblasts (CAFs) represent the most prominent component of the tumor microenvironment (TME). Recent studies demonstrated that CAF are heterogeneous and composed of different subpopulations exerting distinct functions in cancer. CAF populations differentially modulate various aspects of tumor growth, including cancer cell proliferation, extra-cellular matrix remodeling, metastatic dissemination, immunosuppression and resistance to treatment. Among other markers, the Fibroblast Activation Protein (FAP) led to the identification of a specific CAF subpopulation involved in metastatic spread and immunosuppression. Expression of FAP at the surface of CAF is detected in many different cancer types of poor prognosis. Thus, FAP recently appears as an appealing target for therapeutic and molecular imaging applications. In that context, ⁶⁸Ga-labeled radiopharmaceutical-FAP-inhibitors (FAPI) have been recently developed and validated for quantitatively mapping FAP expression over the whole-body using Positron Emission Tomography (PET/CT). In this review, we describe the main current knowledge on CAF subpopulations and their distinct functions in solid cancer, as well as the promising diagnostic and therapeutic implications of radionuclides targeting FAP.

1. Introduction

It is now well-established that tumor development and progression are not strictly dependent on cancer cells, but also controlled by cells of the tumor microenvironment, including fibroblasts, endothelial and immune cells. Cancer-Associated Fibroblasts (CAF) constitute one of the most abundant components of the tumor microenvironment, which contributes to malignancy at several levels. CAF functions in cancer progression have been widely described {Costa, 2014 #38;Pure, 2018 #51;Monteran, 2019 #58;Mhaidly, 2020 #1;Sahai, 2020 #39;Chen, 2021 #52;Mhaidly, 2021 #6;Pradhan, 2021 #53}. Pro-tumorigenic activities of CAF are multiple and include cancer cell proliferation, tumor invasion, angiogenesis, and extracellular matrix (ECM) remodeling. Nevertheless, studies demonstrated that CAF can also prevent cancer cell invasion and exert tumor-suppressive functions {Ozdemir, 2014 #41;Rhim, 2014 #147;Bhattacharjee, 2021 #54}, highlighting the functional duality of the stromal compartment with both pro- and anti-tumorigenic properties. Therefore, many efforts have been achieved to understand this discrepancy and achieve a global characterization of CAF in tumor microenvironment {Mhaidly, 2020 #1;Sahai, 2020 #39;Mhaidly, 2021 #6}. In that context, recent findings demonstrated that CAF are not a homogenous cellular population, but in contrast, that they are composed of several subpopulations. Such heterogeneity can result from various causes, including the numerous precursors of CAF, their reciprocal interactions with cancer cells, as well as any other components of the tumor microenvironment. By combining the study of several markers, including the Fibroblast Activation Protein (FAP) marker, several laboratories all over the world identified a specific FAP⁺ CAF subset in solid cancer. Importantly, this FAP⁺ CAF subpopulation accumulates in cancer of poor prognosis and has been shown to be involved in metastatic spread and immunosuppression in cancer {Pure, 2018 #51;Sahai, 2020 #39;Chen, 2021 #52;Mhaidly, 2021 #6;Pradhan, 2021 #53}. Based on the key pro-tumorigenic roles of the FAP⁺ CAF subpopulation, ⁶⁸Ga-labeled radiopharmaceutical-FAP-inhibitors (FAPI) have recently been developed for whole-body imaging of cancer patients by Positron Emission Tomography (PET/CT) {Calais, 2020 #56;Lindner, 2021 #57}. This review will summarize the recent advances on CAF heterogeneity and their functions in diverse solid cancer types and highlight that FAPI radiotracer paves the way for new diagnostic and therapeutic avenues in cancer.

2. Highlighting CAF heterogeneity in solid cancer

2.1. Identification of different CAF populations by studying specific markers, including FAP, at protein levels

In solid tumors, CAF can be distinguished by morphological criteria and lack of epithelial, hematopoietic, and endothelial cellular markers. In addition, protein levels of specific markers, such as smooth muscle α -actin (SMA), fibroblast activation protein (FAP), platelet-derived growth factor receptor (PDGFR β) and fibroblast-specific protein 1 (FSP1), have been assessed by flow cytometry and immunohistochemistry. While these proteins are either undetected or detected at very low levels in normal fibroblasts, they specifically accumulate in CAF. These markers were first studied individually, and initial evidence that CAF are composed of several cell populations came from these individual protein studies. Myofibroblasts are characterized by high SMA expression (SMA^{High} CAF) and are associated with poor prognosis in many solid cancers, including lung, esophageal, liver, prostate, breast, gastric, colorectal pancreatic and ovarian cancer {Toullec, 2010 #10; Nazareth, 2007 #12; Liu, 2016 #11; Saigusa, 2011 #37; Alcaraz, 2019 #36; Mieulet, 2021 #43}. Notably, SMA^{High} CAF accumulate in aggressive breast cancer, *i.e.* HER2⁺ and triple negative breast cancer (TNBC) {Orimo, 2005 #47; Toullec, 2010 #10; Liu, 2016 #11; Benyahia, 2017 #59}. Analyses of different tumor collections showed associations between PDGFR status in the stroma and patient survival, in line with the role of PDGFR-signaling in tumor drug uptake {Ostman, 2017 #61}. FSP1, also known as S100-A4, is also linked to poor outcome in breast cancer patients {Rudland, 2000 #15}. Finally, high stromal expression of FAP, a serine peptidase with both dipeptidyl peptidase IV and collagenase/gelatinase activity, promotes malignant and invasive behavior of epithelial cancers and correlates with poor prognosis. FAP expression in CAF also correlates with poor breast cancer patient survival, as FAP⁺ CAF promote invasive behavior of epithelial cancer cells and reduce anti-tumor immune response {Costa, 2018 #9; Cremasco, 2018 #13; Bonneau, 2020 #2; Pelon, 2020 #5}.

A big step forward came from the demonstration of CAF heterogeneity by combining the study of several CAF markers. First evidence emerged by studying the differential expression of SMA, PDGFR β , FSP1 and NG2 in pancreatic and breast cancer mouse models {Sugimoto, 2006 #17} and demonstrated that these different markers accumulate in different CAF populations. Interestingly, recent studies in breast and ovarian cancer have succeeded in combining the study of up to 6 different markers altogether by flow cytometry and IHC, including FAP, SMA, PDGFR β , FSP1, Integrin β 1/CD29 and Caveolin1/CAV {Costa, 2018 #9; Givel, 2018 #8}. By this way, 4 CAF populations (named CAF-S1 to -S4) have been identified based on the expression of these markers in breast and ovarian cancer : CAF-S1 (FAP^{High} SMA^{Med-High} FSP1^{Med} PDGFR β ^{Med-High} CD29^{Med} CAV1^{Low}); CAF-S2 (FAP^{Neg} SMA^{Neg} FSP1^{Med-High} PDGFR β ^{Med} CD29^{Low} CAV1^{Low}); CAF-S3 (FAP^{Neg} SMA^{Neg} FSP1^{Neg-Low} PDGFR β ^{Neg} CD29^{Med} CAV1^{Neg}); CAF-S4 (FAP^{Neg-Low} SMA^{High} FSP1^{Low-Med} PDGFR β ^{Low-Med} CD29^{High} CAV1^{Low}) (Table 1) {Costa, 2018 #9; Givel, 2018 #8; Pelon, 2020 #5; Kieffer, 2020 #3}. First

identified by flow cytometry analysis, these 4 CAF subsets have been validated *in situ* by immunohistochemistry on patient samples. Interestingly, these CAF subsets accumulate differentially according to the breast cancer subtypes. CAF-S2 and CAF-S3 are enriched in luminal breast cancer patients and healthy tissues, respectively. Consistent with previous observations, the two myofibroblastic SMA⁺ CAF subpopulations (*i.e.* CAF-S1 and CAF-S4) are enriched in HER2 and TNBC patients. In addition, two subgroups of TNBC patients can be identified, either enriched in CAF-S1 or CAF-S4 {Costa, 2018 #9}. The only use of SMA would not be sufficient to differentiate CAF-S1 and CAF-S4, while CAF-S1 are FAP⁺ CAF in contrast to CAF-S4. The transcriptomic characterization of CAF-S1 and CAF-S4 also pointed out their specificity. Indeed, CAF-S1 gene expression analysis revealed enrichment in ECM and inflammation, while CAF-S4 is driven by a perivascular signature. This is in agreement with their complementary role in breast cancer evolution and metastatic spread {Bonneau, 2020 #2;Pelon, 2020 #5}. Interestingly, CAF heterogeneity can be generalized to other cancers. Indeed, the existence of these 4 CAF subsets has also been uncovered in ovarian cancer {Givel, 2018 #8}. Similarly, CAF-S1 and CAF-S4 accumulate in the HGSOc mesenchymal subtype, which exhibit the worst prognosis among HGSOc patients {Givel, 2018 #8}. Importantly, CAF-S1-enriched cancers, *i.e.* TNBC and mesenchymal HGSOc, exhibit an immunosuppressive environment {Costa, 2018 #9;Givel, 2018 #8;Kieffer, 2020 #3}. Both in breast and ovarian cancer, CAF-S1 are detected preferentially close to epithelial tumor cells (Figure 1). Moreover, the predominant CAF-S1 and CAF-S4 subsets have been observed in other cancer types and mouse models including pancreatic, urothelial, head and neck, lung and colorectal cancers {Sugimoto, 2006 #17;Li, 2017 #18;Lambrechts, 2018 #20;Mariathasan, 2018 #42;Biffi, 2019 #21;Elyada, 2019 #23;Neuzillet, 2019 #22;Strell, 2019 #40;Kieffer, 2020 #3}. Based on key pro-metastatic and immunosuppressive functions of CAF-S1, FAP appears as an appealing target for visualizing and therapeutically targeting CAF-S1.

2.2. Deciphering CAF population heterogeneity by proteomic analysis and single cell RNA sequencing

As mentioned above, among CAF subpopulations, CAF-S1 is characterized by high expression of FAP {Costa, 2018 #9;Givel, 2018 #8}. Interestingly, in breast cancer, the FAP⁺ CAF population can be subdivided according to the podoplanin (PDPN) expression {Cremasco, 2018 #13}. While FAP⁺ PDPN⁺ CAF, characterized by activation of the TGF β signaling pathway, are observed close to T lymphocytes at the tumor border, FAP⁺ PDPN⁻ CAF are mostly perivascular. Likewise, considering SMA expression, two FAP⁺ CAF populations have been identified in pancreatic cancer. FAP⁺ SMA^{High} CAF are defined as myofibroblastic CAF (myCAF), while FAP⁺ SMA^{Low} CAF are characterized by an immunomodulation signature, and thus referred to as inflammatory CAF (iCAF) {Ohlund, 2017 #25;Biffi, 2019 #21;Elyada,

2019 #23}. Preferentially detected at the vicinity of epithelial tumor cells, myCAF exhibit high contractility capacity, through their elevated stress fiber content, ECM synthesis and remodeling. Conversely, iCAF are defined by their production and release of inflammatory molecules, such as IL-6 (Interleukin 6), and are more distal from cancer cells than myCAF {Ohlund, 2017 #25;Bartoschek, 2018 #19;Costa, 2018 #9;Biffi, 2019 #21;Kieffer, 2020 #3;Vickman, 2020 #44}.

Based on these observations, deeper analyses through single-cell RNA sequencing (scRNA-seq) have been performed, in order to have an exhaustive view of CAF heterogeneity. In genetically engineered breast cancer mouse model (MMTV-PyMT), the single-cell analysis of 768 CAF revealed the presence of 3 distinct CAF clusters {Bartoschek, 2018 #19}. Transcriptomic bioinformatic analysis demonstrated their distinct gene expression programs, allowing the description of vascular CAF (vCAF), matrix CAF (mCAF) and developmental CAF (dCAF), confirming previous findings using markers {Costa, 2018 #9;Givel, 2018 #8}. These CAF subsets are also defined by their distinct spatial location in mouse mammary tumors: vCAF are located close to vessels at early stages of tumor development, but within stromal compartment during tumor evolution; mCAF are predominantly detected at tumor periphery and dCAF are close to cancer cells at early tumor states {Bartoschek, 2018 #19}. Similarly, scRNAseq of fibroblasts and vascular mural cells from mouse muscular organs identified inter- and intra-organ heterogeneity amongst fibroblasts, each subtype being localized to discrete anatomical positions {Muhl, 2020 #93}. Another scRNA-seq study performed in TNBC syngeneic mouse model (BALB/c-derived 4T1) revealed 6 different CAF subpopulations, including myofibroblast and inflammatory subpopulations, as well as a subset expressing major histocompatibility (MHC) class II proteins {Sebastian, 2020 #26}. Ly6c1^{High} (lymphocyte antigen 6 complex, locus C1) and SMA^{High} clusters are the most representative clusters in 4T1 mammary tumors, as they contributed to almost 80% of all CAF populations, suggesting their role in tumor growth and progression {Sebastian, 2020 #26}. scRNA-seq from TNBC patients confirmed the existence of two CAF clusters and two perivascular-like (PVL) subpopulations {Wu, 2020 #62}. CAF clusters are characterized by features of myofibroblasts and immunomodulatory molecules and defined as myCAF and iCAF. PVL clusters are distinguished between a differentiated and immature phenotype {Wu, 2020 #62}. Similar iCAF and myCAF clusters were identified in cholangiocarcinoma, the two subpopulations displaying distinct receptor-ligand interactions and contacts with tumor cells {Affo, 2021 #85}. Furthermore, scRNAseq analysis from pancreatic ductal carcinoma reveals a specific CD105/Endoglin⁻ fibroblast lineage that supports anti-tumor immunity {Hutton, 2021 #84}. Recent scRNA-seq coupled with spatial transcriptomic analysis revealed distinct localization of CAF subpopulations in breast cancer patients, and identified stromal-immune niches uncovering potential insights into stromal-mediated immunoregulation {Wu, 2021 #64}.

Comprehensive scRNAseq analysis of CAF at several time points along breast tumor progression in mice uncover distinct CAF subpopulations, which change over time and in metastases from an immunoregulatory program to wound-healing and antigen-presentation programs {Friedman, 2020 #96}. Finally, integration of a large series of scRNA-seq datasets, composed of 230 000 fibroblasts of mouse and human origins, showed similarities in terms of phenotypes and transcriptomic profiles between the two species, both in healthy and diseased tissues, thereby revealing a cross-species and pan-tissue fibroblast lineage in health and disease {Buechler, 2021 #63}.

In addition to scRNAseq from total CAF subpopulations, recent studies have focused their analysis on FAP⁺ CAF, as these cells exhibit both pro-metastatic and immunosuppressive functions (see below). Importantly, heterogeneity within the FAP⁺ CAF population has also been demonstrated in breast, lung and head and neck cancer patients by one of the most exhaustive scRNAseq studies of this CAF population published until now {Kieffer, 2020 #3}. scRNAseq analysis of more than 18 000 FAP⁺ CAF revealed the presence of 8 distinct FAP⁺ CAF clusters in breast cancer {Kieffer, 2020 #3}. Among them, 5 are referred to as myofibroblastic (myCAF) clusters and 3 to as inflammatory (iCAF) clusters {Kieffer, 2020 #3}, as previously identified in pancreatic cancer {Ohlund, 2017 #25;Biffi, 2019 #21;Elyada, 2019 #23}. Based on their transcriptomic profile, the 5 myCAF clusters are defined by ECM proteins (ECM-myCAF), Transforming Growth Factor β signaling pathway (TGF β -myCAF), wound healing (wound-myCAF), interferon $\alpha\beta$ (IFN $\alpha\beta$ -myCAF) and acto-myosin pathway (acto-myCAF) and the 3 iCAF clusters by detoxification pathway (detox-iCAF), interleukin-signaling (IL-iCAF) and interferon γ (IFN γ -iCAF). Interestingly, the existence of the 5 most abundant FAP⁺ CAF clusters (ECM-myCAF, TGF β -myCAF, wound-myCAF, detox-iCAF and IL-iCAF), representing 91% of all FAP⁺ CAF single cell analyzed, have been validated in breast cancer samples by multicolor flow cytometry using specific markers {Kieffer, 2020 #3}. All FAP⁺ CAF clusters can be detected in all tested breast cancer patients but in different proportions. The proportion of these FAP⁺ CAF clusters vary from one patient to the other, showing an inter-patient heterogeneity {Kieffer, 2020 #3}. This study also demonstrated that luminal breast cancer patients are enriched in ECM-myCAF, TGF β -myCAF and wound-myCAF, whereas TNBC patients show a higher proportion of detox-iCAF and IL-iCAF {Kieffer, 2020 #3}. Interestingly, applying those transcriptomic signatures on publicly available datasets of head and neck (HNSCC) and non-small cell lung cancers (NSCLC) demonstrated the existence of these FAP⁺ CAF clusters in several cancer types. Notably, a scRNA-seq study of CAF coming from pancreatic cancer patients (PDAC) revealed similarities with myCAF FAP⁺ CAF clusters and notably highlighted the presence of an ECM-enriched FAP⁺ CAF cluster {Dominguez, 2020 #45}.

2.3. CAF origin and plasticity

CAF heterogeneity in tumors raise the question of their origin and plasticity during tumor progression. CAF origin in tumors have been questioned in the past few years, and several studies reported that they can originate from resident tissue fibroblasts, mesenchymal stem cell from bone marrow, adipose tissue, endothelial cells, pericytes or adipocytes {Zeisberg, 2007 #154;Quante, 2011 #155;Kidd, 2012 #156;Bochet, 2013 #163;Rhim, 2014 #147;Hosaka, 2016 #157;Strong, 2017 #158;Raz, 2018 #153;Strell, 2019 #40;Mhaidly, 2020 #1}. In breast cancer, the differentiation of resident fibroblasts into myofibroblasts is induced by tumor cells through their release of reactive oxygen species (ROS) which induce CXCL12/CXCR4, TGF β and NOTCH signaling pathway activation {Toullec, 2010 #10;Costa, 2014 #38;Gentric, 2017 #159;Mhaidly, 2020 #1}. Interestingly, CAF diversity in breast cancer can rely on distinct cells of origin. Indeed, consistent with their location, vCAF could originate from invading perivascular cells, while mCAF could be derived from normal resident fibroblast as they express common markers {Bartoschek, 2018 #19}. Finally, dCAF share similarities with epithelial tumor cells in their transcriptomic profiles, suggesting that EMT might give rise to this CAF population {Bartoschek, 2018 #19}. CAF plasticity in tumors could also contribute to their heterogeneity, as CAF populations can derived from each other in presence of specific stimuli. For instance PDAC-derived iCAF can be converted into myCAF, when cultured in monolayer or treated with TGF β {Boyd, 2021 #164;Ohlund, 2017 #25}. Moreover, the co-culture of ECM-myCAF isolated from breast tumor together with CD4⁺ CD25⁺ T lymphocytes increase the expression of LAMP5 at ECM-myCAF surface. LAMP5 is a specific marker of the TGF β -myCAF cluster, indicating that in presence of T lymphocytes, ECM-myCAF can give rise to TGF β -myCAF {Kieffer, 2020 #3}. Finally, to go deeper, cutting edge single-cell technologies coupled with relevant bioinformatic programs represent the most promising approach to get insights into CAF diverse origins and their dynamic plasticity during tumor progression.

In conclusion, heterogeneity of CAF subsets, especially the FAP⁺ CAF subpopulation, has been widely studied in the last few years. The existence of distinct CAF populations and subpopulations in several cancer types raised clinical concerns regarding their role in tumor progression and resistance to treatments (see below in sections 3.2 to 3.4 and Figure 2). Based on accumulation of FAP⁺ CAF in cancers of poor prognosis, characterizing the multiple functions of this specific population has been of particular interest in the past years.

3. Functions of FAP⁺ CAF subpopulations in solid cancer

3.1. *The Fibroblast Activation Protein*

The Fibroblast Activation Protein is a 760 amino acid long type II transmembrane glycoprotein encoded by the FAP gene. It is a non-classical serine protease, the active site of which being localized in the extracellular part of the protein. It has both dipeptidyl peptidase and endopeptidase activities. The dipeptidyl peptidase activity of FAP cleaves two amino acids off the N-terminus of a protein, after a proline residue. The endopeptidase activity of FAP makes it possible to cleave more than two amino acids from the N-terminus of a protein at the post-proline bond {Hamson, 2014 #142}. The fundamental biology of FAP is not fully elucidated yet. Known physiological endopeptidase substrates of FAP are type α 2-antiplasmin {Lee, 2006 #143} and fibroblast growth factor-21 (FGF-21) {Dunshee, 2016 #144} but FAP preponderantly degrades proteins of the extracellular matrix (ECM), including type I collagen (CN-I), CN-III, CN-V, fibrillin-2 and ECM-1. In addition, FAP has also been demonstrated to have a “downstream degradome” effect {Koczorowska, 2016 #146;Zhang, 2019 #145} affecting laminins, fibronectin, periostin, cofilin-1, cathepsin-B, fibrillin-1, fibulin-1, serpin-H1, lysyl oxidase homolog 1 (LOX-L1), Neural Cell Adhesion Molecule NCAM-1 as well as the ECM degrading enzymes matrix metalloproteinases MMP-2 and MMP-3 and MMP inhibitors TIMP-1 and TIMP-2. Experimental data {Zhang, 2019 #145} suggest that FAP increases ECM proteolysis and plays a significant role in the regulation of ECM degradation, either directly or indirectly, making it a key actor of the remodeling of the pericellular environment.

3.2. *Role of FAP⁺ CAF in metastatic spread*

The role of FAP⁺ CAF on metastatic spread implicates a global action within the tumor microenvironment and on cancer cells. Indeed, FAP⁺ CAF induce a pro-inflammatory and pro-angiogenic microenvironment, notably by the secretion of pro-inflammatory cytokines, such as IL-6 and pro-angiogenic factors, such as the vascular endothelial growth factor A (VEGF-A) {Johnson, 2018 #50;Jones, 2018 #49}. FAP⁺ CAF interplay with cancer cells involves the CXCL12-CXCR4 (C-X-C Motif Chemokine Ligand 12 / Receptor 4) axis at least in two pro-tumorigenic activities: cancer cell proliferation and metastatic spread {D'Arcangelo, 2020 #73;Patel, 2020 #72;Sahai, 2020 #39;Mhaidly, 2021 #6}. In breast cancer, the chemokine CXCL12, secreted by FAP⁺ CAF promote angiogenesis and directly induce breast cancer cell proliferation, which expresses its cognate receptor CXCR4 at their surface membrane {Orimo, 2005 #47;Toullec, 2010 #10}. This feature can be generalized to other carcinomas, as reported in esophagogastric, endometrial and pancreatic cancers {Feig, 2013 #28;Sugihara, 2015 #27;Teng, 2016 #29;Kashima, 2019 #74;Francescone, 2021 #94}. CAF can also promote cancer cell proliferation, as well as increase stemness features in colon, pancreatic and breast cancer {Orimo, 2005 #47;Sahai, 2020 #39}. In solid cancer, such as breast and ovarian cancer, myofibroblastic FAP⁺ CAF induce cancer cell metastatic spread into secondary sites by

initiating tumor cell epithelial-to-mesenchymal transition (EMT), promoting their migration, and increasing their invasive abilities by ECM remodeling {Zhou, 2014 #76;Attieh, 2017 #82;Lefort, 2017 #46;Bonneau, 2020 #2;Pelon, 2020 #5;Bota-Rabassedas, 2021 #86;Mieulet, 2021 #43}. Reciprocally, direct contacts between cancer cells and CAF trigger the expression of a wide range of inflammatory molecules by fibroblasts {Arwert, 2020 #92}. ECM remodeling by CAF through the production of collagen and matrix metalloproteinases (MMP) has been shown to increase tumor stiffness. In ovarian cancer, the more the TME is enriched in myofibroblasts, the more the tumor is stiff {Mieulet, 2021 #43}. Therefore, mechano-transduction pathways, such as YAP/TAZ, promote CAF pro-tumorigenic activities, including cancer cell proliferation, invasion and migration {Nagelkerke, 2015 #161;Bertero, 2019 #162}.

In breast cancer, although 4 CAF subsets were detected in metastatic axillary lymph nodes, the two myofibroblastic CAF subsets, *i.e* FAP⁺ CAF/CAF-S1 and CAF-S4, are the most abundant populations and correlate with the accumulation of cancer cells, suggesting their major role in cancer cell dissemination {Pelon, 2020 #5}. These two myofibroblastic subpopulations exhibit complementary functions in metastatic dissemination, by attracting cancer cells and promoting their EMT through CXCL12 and TGF β signaling on the one hand, and by promoting ECM remodeling and cancer cell invasion by NOTCH signaling pathway on the other {Pelon, 2020 #5}. In addition, consistent with luminal breast cancer recurrent metastatic sites in bones, FAP⁺ CAF pro-metastatic activity also involves the CDH11/osteoblast cadherin {Bonneau, 2020 #2}. FAP⁺ CAF have been detected in higher proportion in the peripheral blood of metastatic than of localized breast cancer patients {Ao, 2015 #77;Solano-Iturri, 2020 #78}. In line with that, FAP⁺ CAF have been associated with microvessel and lymphatic vessel density in lung cancer {Chen, 2018 #79}. In conclusion, considering that specific CAF populations can have distinct functions emphasizes the importance of deeply characterizing CAF heterogeneity to find new therapeutic approaches and tackle their pro-tumorigenic effect. FAP⁺ CAF density, assessed by FAP pattern, should be considered as a novel marker for disease prognosis (Figure 2).

3.3. Role of CAF subpopulations in immunosuppression

Based on CAF heterogeneity in solid cancer, the impact of CAF subpopulations on the immune compartment has been particularly investigated {De Jaeghere, 2019 #95;Mhaidly, 2021 #6}. In pancreatic cancer, immunosuppressive functions of FAP⁺ CAF involve the CXCL12 chemokine, which attracts and retains CD4⁺ and CD8⁺ T lymphocytes within the stromal compartment {Ene-Obong, 2013 #33;Feig, 2013 #28;Gorchs, 2019 #34;Hornburg, 2021 #87}. Moreover, CAF extracted from pancreatic tumors increase the expression of key immune checkpoints, such as TIM-3, PD-1, CTLA4 and LAG-3 in T lymphocytes *in vitro*, through the action of prostaglandin E2 {Gorchs, 2019 #34}. In addition, CD105/Endoglin

expression differentiates two functionally distinct fibroblast lineages in pancreatic adenocarcinoma, which are both identified in murine and human tissues. Whereas CD105⁺ pancreatic CAF are permissive for tumor growth, CD105⁻ fibroblasts are highly tumor suppressive by regulating a functional adaptive immunity {Hutton, 2021 #84}. In breast and ovarian cancers, FAP⁺ CAF/CAF-S1 induce an immunosuppressive tumor microenvironment by directly modulating CD4⁺ T lymphocytes content, phenotype and function through a multistep mechanism {Costa, 2018 #9;Givel, 2018 #8}. Indeed, FAP⁺ CAF attract CD4⁺ CD25⁺ T lymphocytes through CXCL12 and retain them through long-term interactions involving OX40L, PD-L2 and JAM2 {Costa, 2018 #9;Fearon, 2014 #31;Givel, 2018 #8}. Moreover, FAP⁺ CAF promote CD4⁺ CD25⁺ T lymphocyte differentiation into FOXP3⁺ regulatory T cells through B7H3, CD73 and DPP4 {Costa, 2018 #9;Givel, 2018 #8}.

The characterization of FAP⁺ CAF heterogeneity in breast cancer through scRNA-seq analysis allowed to go deeper in the identification of specific FAP⁺ CAF clusters, *i.e.* ECM-myCAF, TGFβ-myCAF and wound-myCAF, involved in immunosuppression {Kieffer, 2020 #3}. The immunosuppressive activity of the TGFβ signaling pathway has been showed to inhibit proliferation and cytotoxic activity of CD8⁺ T lymphocytes, and to reduce survival of effector CD8⁺ T cells {Lakins, 2018 #68;Takahashi, 2015 #70;Thomas, 2005 #69}. Moreover, the content in ECM-myCAF and TGFβ-myCAF clusters are correlated with the infiltration of PD-1⁺, CTLA-4⁺ and TIGIT⁺ CD4⁺ T lymphocytes in breast cancer {Kieffer, 2020 #3}. Accordingly, ECM-myCAF upregulate PD-1 and CTLA4 protein levels at the surface of FOXP3⁺ regulatory T cells, demonstrating their ability to induce an immunosuppressive microenvironment {Kieffer, 2020 #3}. In colon, lung, breast and pancreatic cancers, CAF inhibit T cell functions by their direct interaction through the PD-L1 or PD-L2 / PD-1 receptor axis {Pinchuk, 2008 #67;Costa, 2018 #9; Lakins, 2018 #68;Gorchs, 2019 #34}. As shown here, CAF can display direct immunosuppressive functions on T cells, but could also have an indirect mode of action through tumors cells. Indeed, the release of CXCL5 by CAF induces the expression of PD-L1 at the surface of melanoma and colorectal mouse tumor cells, which subsequently could decrease T-cell activity {Li, 2019 #65}. Beside direct effects of CAF on T lymphocytes, CAF can also contribute to the establishment of an immunosuppressive environment in lung cancer, by excluding immune cells through ECM production and remodeling, thereby serving as a physical barrier {Salmon, 2012 #32}.

In addition to their immunosuppressive function on T lymphocytes, CAF also affect the cytotoxic abilities of Natural Killer (NK) cells {Mhaidly, 2020 #1}. Indeed, CAF derived from metastatic melanoma and colorectal cancer have been shown to suppress NK cells functions by decreasing the expression of the NK activating receptors NKp44, NKp30 and DNAM-1 as well as the formation of cytolytic granules {Balsamo, 2009 #152;Li, 2013 #148}. Indeed, co-culture of CAF from colorectal and pancreatic cancer together with NK cells reduces the

expression of perforin and granzyme B cytolytic proteins by NK {Huang, 2019 #151;Li, 2013 #148;Malchiodi, 2021 #150}. In NSCLC, CAF have a dual immunosuppressive function on NK cells, as CAF both promote an inhibitory NK phenotype through an increased expression of the inhibitory receptor NKG2A and inhibit NK cell activation by decreasing their proliferation, cytotoxic ability, and expression of the stimulatory receptor NKG2D {Yang, 2020 #149}.

Altogether, CAF and especially FAP⁺ CAF modulate the immune content by hijacking immune cells functions to induce an immunosuppressive TME. Clinically, this must be of high relevant to predict some cancer treatment efficacy, such as that of immunotherapy (Figure 2).

3.4. Role of CAF subpopulations in resistance to immunotherapy

Given the role of CAF in shaping an immunosuppressive microenvironment, it seems likely that we must account for it to better understand resistance to immunotherapy {Barrett, 2020 #80}. Distinct T cell infiltration patterns, *i.e.*, immune infiltrated, excluded, and desert, result in different responses to cancer immunotherapies {Bagaev, 2021 #89;Hornburg, 2021 #87}. A recent dissection at high-resolution through scRNAseq analysis of 15 ovarian tumors showed that immune-infiltrated and -excluded tumors differ markedly in fibroblast subsets {Hornburg, 2021 #87}. In line with the impact of the TGF β signaling pathway in immunosuppression driven by CAF, this pathway has been associated with low immunotherapy response in NSCLC, melanoma, metastatic urothelial and colon cancer {Mariathasan, 2018 #42;Tauriello, 2018 #71;Kieffer, 2020 #3;Hu, 2021 #90}. Interestingly, in colorectal and in breast cancer mouse models, blocking TGF β signaling re-sensitize tumor cells to anti-PD1-PD-L1 therapy by remodeling CAF dynamics and restoring cytotoxic T cell response {Tauriello, 2018 #71;Grauel, 2020 #91}. Furthermore, the analysis of public data from metastatic melanoma and lung cancer patient treated with anti-PD-1 therapy revealed that 3 FAP⁺ CAF clusters (ECM-myCAF, TGF β -myCAF and wound-myCAF) are enriched at diagnosis in non-responder patients {Kieffer, 2020 #3}. Therefore, these CAF clusters are indicative, at the time of diagnosis, of primary resistance to immunotherapies. This underlines the need to have an integrating and comprehensive knowledge of TME populations and especially the composition of CAF content within tumors to be able to predict and tackle immunotherapy resistance (Figure 2).

4. Medical applications: diagnostic tool and targeted therapies

4.1. Development of FAP ligands for non-invasive imaging of FAP expression

Targeting FAP overexpressed in a specific population of CAF has been investigated for in vivo molecular imaging with Single Photon Emission Tomography and PET for more than two decades using antibodies or inhibitor molecules {Lindner, 2019 #126}. Yet, this quest has

reached a turning point in 2018 with the development of a series of low-molecular-weight FAP quinoline-based inhibitors (FAPI) labelled with positron emitting ^{68}Ga , thus amenable to whole-body quantitative PET imaging.

These ^{68}Ga -labelled FAPI compounds were based on a small molecule enzyme FAP inhibitor with high affinity {Jansen, 2013 #101}. The first two developed compounds, FAPI-01 and FAPI-02, showed encouraging specific binding to human and murine FAP with a fast and almost complete cellular internalization. Yet, FAPI-02 had superior pharmacokinetic and biochemical properties than FAPI-01 and was demonstrated to display a high intratumoral uptake and fast body clearance, producing high-contrast PET images in patients with metastasized pancreatic cancer, breast cancer, and locally advanced lung adenocarcinoma {Loktev, 2018 #127}. Still, FAPI-02 tumor retention was too short to consider any theranostic application, *i.e.* where the compound can be used both for diagnostic imaging and for molecular radiotherapy. Lindner et al. therefore compared 15 variants of FAPI in terms of binding, internalization, and efflux, in cells expressing human and murine FAP as well as CD26 and identified FAPI-04 as a promising radiotracer candidate for both diagnostic imaging and theranostics {Lindner, 2018 #104}. Further FAPI variants have then been developed to further improve FAP binding and optimize clinical imaging while enabling possible theranostic applications, with FAPI-46 currently being one of the most promising ^{68}Ga -FAPI tracers in terms of binding, retention time, fast body clearance {Loktev, 2019 #103}, and dosimetry {Meyer, 2020 #128}. FAPI-46 yielded remarkable PET image quality with high tumor-to-organ/blood ratio as soon as 10 min after injection {Ferdinandus, 2021 #105}. In addition, Mona et al. demonstrated that ^{68}Ga -FAPI-46 PET biodistribution faithfully reflects FAP expression as revealed by immunohistochemistry on a pan-cancer human tissue microarray in 141 patients and 14 different types of cancer (correlation coefficient=0.781 between FAP immunohistochemistry score and ^{68}Ga -FAPI-46 maximum standardized uptake value across cancer and non-cancer tissues; $p < 0.001$) {Mona, 2021 #106}.

Beyond ^{68}Ga -FAPI, FAPI compounds labelled with positron emitter Fluorine 18 (^{18}F) are extremely appealing because of easier large-scale production compared to ^{68}Ga -FAPIs, which could further facilitate their use in clinical routine. Several ^{18}F -labelled tracers targeting FAP have thus been recently described. For instance, a FAP inhibitor using a NOTA chelator, called FAPI-74, has been labelled with ^{68}Ga or ^{18}F -AlF (Aluminium Fluoride) and offers high contrast and low radiation burden {Giesel, 2021 #107}. Another Aluminium- ^{18}F -labelled NOTA-conjugated FAPI, named Al ^{18}F -NOTA-FAPI, has been developed for FAP-targeted tumor imaging and showed excellent imaging quality and tumor detection efficacy in 10 cancer patients including lung cancer, pancreas cancer, colorectal cancer, prostate cancer and lymphoma patients {Wang, 2021 #108}. A ^{18}F -labeled glycosylated FAPI (^{18}F -FGlc-FAPI) has

also been described and evaluated in nude mice bearing HT1080hFAP tumors or U87MG xenografts, yielding a 15% radioactivity yield and a high radiochemical purity of more than 99% {Toms, 2020 #109}. Similar results have been found by Hu et al. using ^{18}F -FAPi-42 PET/CT, which exhibits comparable lesion detectability to ^{68}Ga -FAPi-04 PET/CT {Hu, 2021 #110}.

4.2. Clinical evaluation of different radiotracer candidates for in vivo imaging of FAP expression

Most groups have tested their radiotracer candidates for in vivo mapping of FAP expression in different preclinical or clinical setups, which complicates the comparison of the radiotracer performance. The FAP radiotracer best suited to such mapping still remains to be agreed on. Most clinical oncology studies involving more than 10 patients have focused on the ^{68}Ga -labeled FAPi-04 and FAPi-46. Altogether, these studies have already involved hundreds of patients {Sollini, 2021 #129} in various clinical settings (eg, head and neck, glioblastoma, pancreatic carcinoma, oesophageal cancer, hepatic nodules, gastrointestinal malignancies, peritoneal carcinomatosis) and have largely demonstrated the clinical potential of FAPi PET/CT. Depending on the study, the tracer performances have been compared either with contrast-enhanced Computed Tomography, with Magnetic Resonance Imaging, with endoscopy or, most often, with ^{18}F -fluorodeoxyglucose (FDG) PET/CT. In contrast to FDG PET/CT, FAPi PET/CT does not require fasting, and imaging can be performed as early as 10 minutes after injection {Ferdinandus, 2021 #105}. Tumor lesions greater than a few millimeters in size are systematically associated with a supporting stroma, which volume exceeds that of the cancer cells. Therefore, PET imaging of the FAP+ CAF expressed in the stroma might be more sensitive than PET imaging of the glucose metabolism by tumor cells. Given the absence of FAP expression in fibroblast-devoid tissues, the tumor-to-surrounding tissue contrast might also exceed that observed when using ^{18}F -FDG PET.

Many recent studies have thus investigated the potential of FAPis to compete with or even outperform FDG for several clinical indications, including diagnosis/staging {Kratochwil, 2019 #115}, treatment response assessment {Dendl, 2021 #116}, radiotherapy planning {Zhao, 2021 #117} or theranostics {Altmann, 2021 #83}. For instance, Chen et al. showed the superiority of ^{68}Ga -DOTA-FAPi-04 PET/CT over ^{18}F -FDG PET/CT for initial staging and recurrence detection in 75 patients with 12 different tumor types, including breast cancer {Chen, 2020 #118}. The main advantages of the ^{68}Ga -DOTA-FAPi-04 PET/CT images were their high contrast, high sensitivity for lymph node, bone or visceral metastases, as well as brain or peritoneal lesions. Such findings have been confirmed in a specific cohort of 20 metastatic breast cancer patients {Komek, 2021 #119}. A recent comprehensive analysis of the published literature {Sollini, 2021 #129} showed that the sensitivity of ^{68}Ga -FAPi for the diagnosis of the primary tumor reached 1 (95% confidence interval CI 0.98-1.00) without heterogeneity among studies. The sensitivity for the detection of distant tumor metastases was

also very high (0.93 with CI 0.88-0.97). ⁶⁸Ga-FAPI outperformed MRI and ¹⁸F-FDG PET/CT to delineate skull base and invasive intracranial disease in 15 patients with nasopharyngeal carcinoma {Qin, 2021 #132}. Yet, the sensitivity of ⁶⁸Ga-FAPI PET/CT in detecting nodal involvement in various cancer types is heterogeneous among studies, and sometimes lower than that of ¹⁸F-FDG PET/CT, with values varying from 59% to 100% {Sollini, 2021 #129}. This might be related to various cell enrichment within lymph nodes compared to the primary tumor, as reported in breast cancer, possibly caused by different transcriptomic programs in fibroblast located in different tissues {Cremasco, 2018 #13; Pelon, 2020 #5}.

Last, pre-treatment ¹⁸F- or ⁶⁸Ga-FAPI PET/CT imaging might also be a relevant assay to predict patient response to FAP-blockade therapies, alone or in combination with other tumor therapies {Xin, 2021 #133}, using a concept similar to that investigated to predict response to immune checkpoint inhibitors targeting PD-1/PD-L1 {Nienhuis, 2021 #134}. Whether FAPI PET/CT can be used to provide a companion biomarker able to predict response to novel FAP-blockade therapy remains to be investigated.

4.3 FAP-based therapeutic regimens

Based on the previously described properties, FAP appear as very promising targets for cancer therapy. Various FAP-based therapeutic approaches have been described, including radionuclide-based approaches (see section 4.4), FAP-activated prodrugs (<https://pubmed.ncbi.nlm.nih.gov/22911669/>), FAP antibodies conjugated with toxins, immunomodulatory cytokines or costimulatory molecules (<https://pubmed.ncbi.nlm.nih.gov/34490078/>), FAP- bispecific T-cell engager-encoding virus (<https://pubmed.ncbi.nlm.nih.gov/30449733/>) and anti-FAP CAR-T cells (<https://pubmed.ncbi.nlm.nih.gov/29748183/>).

Preclinical studies with anti-FAP antibodies showed encouraging results in multi-drug resistant colon and breast cancer models (PMID: 16794736) and in immunodeficient mice with stroma-rich models (PMID: 18628473). However, the first phase II trial conducted in 17 patients with metastatic colorectal cancer failed to confirm these promising preclinical findings (PMID: 12624517).

The specific depletion of FAP-positive CAFs led to slower tumor growth (PMID: 24277834, PMID: 24778279, PMID: 27036971). Furthermore, FAP-targeting strategies gave best results when combined with other therapeutic options such as chemotherapy (PMID: 28004985), vaccines (PMID: 27119119), antibodies (PMID: 27037412), immunotoxins (PMID: 26334777).

FAP is an enzyme and may therefore be used as a target for drugs or prodrugs. As an example, prodrugs have been used using FAP substrates coupled to cytotoxic drugs, as well as doxorubicin (PMID: 28063352). The prodrug can thus be specifically and efficiently

activated in tumor tissue of mice (PMID: 22323494). These results have only been obtained in preclinical studies and further clinical evaluation should be supported and encouraged. Moreover, selective FAP inhibitors could also be used (PMID: 23594271). Eager reported a phase II trial evaluating talabostat, an orally available amino boronic dipeptide that specifically inhibits dipeptidyl peptidases including FAP, in combination with docetaxel in 42 advanced non-small cell lung cancer patients (PMID: 19501491). While talabostat remained very well tolerated, only 3 patients (7 %) demonstrated a major clinical benefit. FAP-specific inhibitors might also be considered for the development of novel radiopharmaceuticals. To the best of our knowledge, there are currently no biomarkers that would indicate the most appropriate FAP-targeted therapeutic regimen. Further prospective clinical studies are needed to explore the full potential of these different options. Still, FAP expression is a prerequisite for successful FAP-targeted treatment, hence the relevance of ^{18}F - or ^{68}Ga -FAPI PET/CT imaging to assess the FAP expression over the whole-body.

4.4 Focus on molecular radiotherapy targeting FAP

FAP-targeted molecular radiotherapy can deliver ionizing radiations to FAP and also to surrounding cancer cells through crossfire effects. Molecular radiotherapy {Sgouros, 2020 #135} has been proven effective in other contexts, such as treatment of metastatic castration-resistant prostate cancer by targeting PSMA using Lutetium-177 ^{177}Lu PSMA {Hofman, 2021 #120}. ^{177}Lu is a β - emitter with a short average range of 0.6 mm in soft tissues, with a long half-life (6.7 days) well compatible with the pharmacokinetics of antibodies and peptides used for molecular radiotherapy. Lindner's team was the first to report a single therapeutic application of 2.9 GBq ^{177}Lu -labeled FAPI-04 in a breast cancer patient, which led to a significant reduction of pain medication in this patient {Lindner, 2018 #104}. Several authors confirmed the safety and feasibility of molecular radiotherapy in a few cancer patients using ^{177}Lu -FAPI-04 (n=4pts, 1 cycle) {Kuyumcu, 2021 #136}, with a reasonably low absorbed dose to organs at risk, but also with ^{177}Lu -FAPI-46 (n=18pts, 2 cycles) {Assadi, 2021 #124}. Baum et al. presented first-in-human results using ^{177}Lu -FAP-2286 for molecular radiotherapy in 11 patients with advanced pancreatic, breast, rectal and ovarian carcinomas, who displayed a significant uptake on previously performed ^{68}Ga -FAP-2286/-FAPI-04- PET/CT {Baum, 2021 #125}. In this retrospective study, the tolerance of molecular radiotherapy with ^{177}Lu -FAP-2286 was acceptable and demonstrated a modest clinical benefit with pain reduction in 3 patients. Other therapeutic FAPI agents labelled with ^{177}Lu are being developed and investigated in terms of biodistribution, pharmacokinetics and/or dosimetry in animal models and patients with various types of cancer {Ballal, 2021 #137; Xu, 2021 #139; Zhang, 2021 #138}.

Molecular radiotherapy can also be performed using Yttrium 90 as a radionuclide. ^{90}Y is another β - emitter with a 64.2 hour half-life, and an approximate emission range in soft

tissues of 5.3 mm, well suited for uniformly irradiating tumors. Yttrium 90 ⁹⁰Y-labeled FAPI-46 treatment has been reported for two patients with ovarian cancer and pancreatic cancer who received ⁹⁰Y-FAPI-46 as last-line therapy {Lindner, 2020 #122}. Ferdinandus et al. also investigated molecular radiation therapy with 2 cycles of ⁹⁰Y-FAPI-46 in 9 metastatic patients (sarcoma or pancreatic cancer) and reported a low rate of adverse events, a low absorbed dose to organs at risk and finally, disease control in nearly one-third of patients {Ferdinandus, 2021 #123}. Eiber et al treated a metastatic non-small cell lung cancer patient with one cycle of ⁹⁰Y-FAPI-04 (7.4 GBq) combined with an immune checkpoint inhibitor blocking the programmed cell death receptor (PD-1) (Pembrolizumab). Despite a dissociated treatment response with some shrinking lesions together with emerging new lesions, the patient had a clinically meaningful improvement {Eiber M., 2021 #141}. These findings open new insights for the blockade of FAP in combination with another therapy. Given the immunosuppressive properties of FAP-expressing CAFs, possible synergetic effects of using FAP-targeted radiation therapy in combination with ICIs deserve investigations.

Other radionuclides can be considered for FAP targeted molecular radiotherapy, such as Iodine 131 (¹³¹I), a β^- emitter that has been used for decades to treat thyroid cancer, or Astatine 211 (²¹¹At), an alpha particle emitter, that might be a lower toxicity alternative to Iodine 131 {Ma, 2021 #140}.

Even if the most effective diagnostic and therapeutic compounds for targeting FAP remain to be determined, the combined use of a ⁶⁸Ga or ¹⁸F-labelled imaging agent with its ¹⁷⁷Lu or ⁹⁰Y counterpart offers highly promising opportunities for developing theranostic approaches: PET imaging can be used to image the presence and biodistribution of FAP+ CAF and molecular radiotherapy can deliver radiations directly to CAF expressing FAP. In addition, almost all radionuclides used in molecular radiotherapy also emit photons that can be detected using Single Photon imaging {Lindner, 2018 #104}, thus enabling the non-invasive mapping of the biodistribution of the therapeutic agent after treatment and subsequent image-based, patient-specific dosimetry, for assessing the dose that was actually delivered to the FAP-expressing CAF, towards a personalized treatment design and monitoring.

5. Conclusion

Most advanced techniques, from single cell analysis, spatial transcriptomics up to whole-body molecular imaging and molecular radiotherapy, offer unique opportunities to elucidate the role of fibroblasts in cancer development and resistance to treatment, and to propose and customize treatment targeting FAP expression. The multi-scale approach enabled by the combination of these tools might also be key to understand the heterogeneity of the tumor (micro)environment and get a comprehensive and quantitative view of its impact

on tumor cell evolution before and during therapy. It is expected that leveraging these complementary insights will ultimately yield new treatment strategies.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Conflicts of Interest

F.M.-G. received research support from Innate-Pharma, Roche and Bristol-Myers-Squibb (BMS). A.P. declares no potential conflict of interest. F-C.B. received research support from Roche. I.B. receives research support from Siemens Healthineers, GE Healthcare and Dosisoft.

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Table and Figures





	FAP	SMA	FSP1	PDGFR	CD29	CAV1
CAF-S1 	+++	++/+++	++	++/+++	++	+
CAF-S2 	-	-	++/+++	++	+	+
CAF-S3 	-	-	-/+	-	++	-
CAF-S4 	-/+	+++	+/>++	+/>++	+++	+

Table 1: CAF subsets defined by the expression of 6 different markers including FAP, SMA, FSP1, PDGR β , CD29 and CAV1.

FAP, SMA, FSP1, PDGR β , CD29 and CAV1 protein levels have been first identified by flow-cytometry and further validated by IHC (+++: high protein level, ++: medium protein level, +: low protein level, -: undetected).

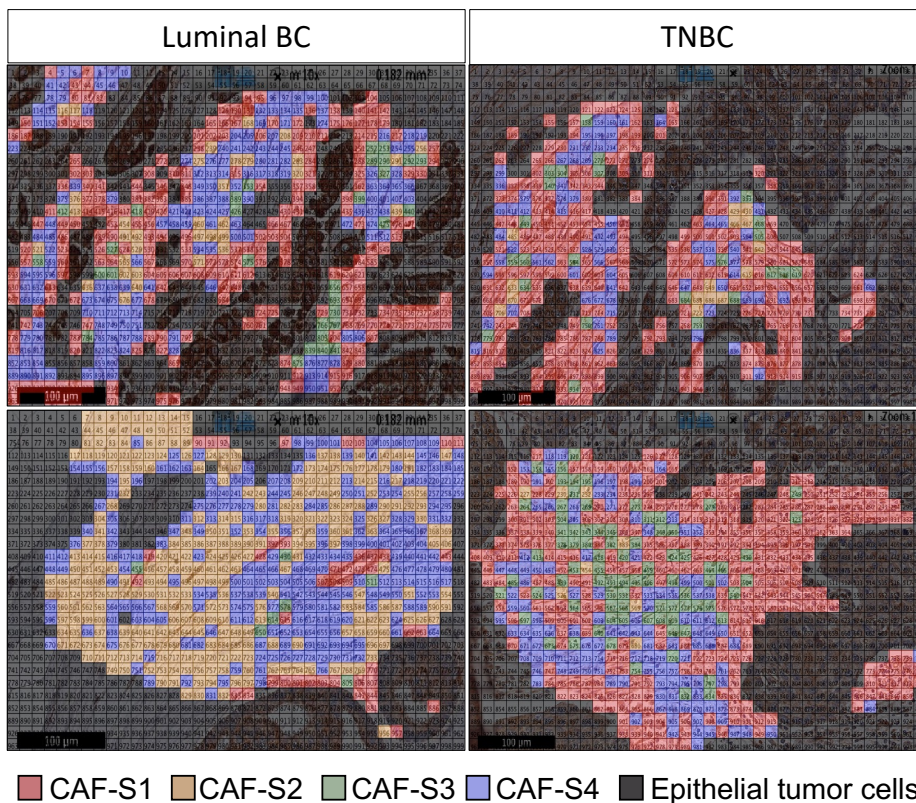


Figure 1: CAF-S1 are preferentially detected close to epithelial tumor cells in luminal and TNBC.

Maps of CAF subsets (red: CAF-S1, orange: CAF-S2, green: CAF-S3 and blue: CAF-S4) at cellular scale in luminal and TNBC sections.

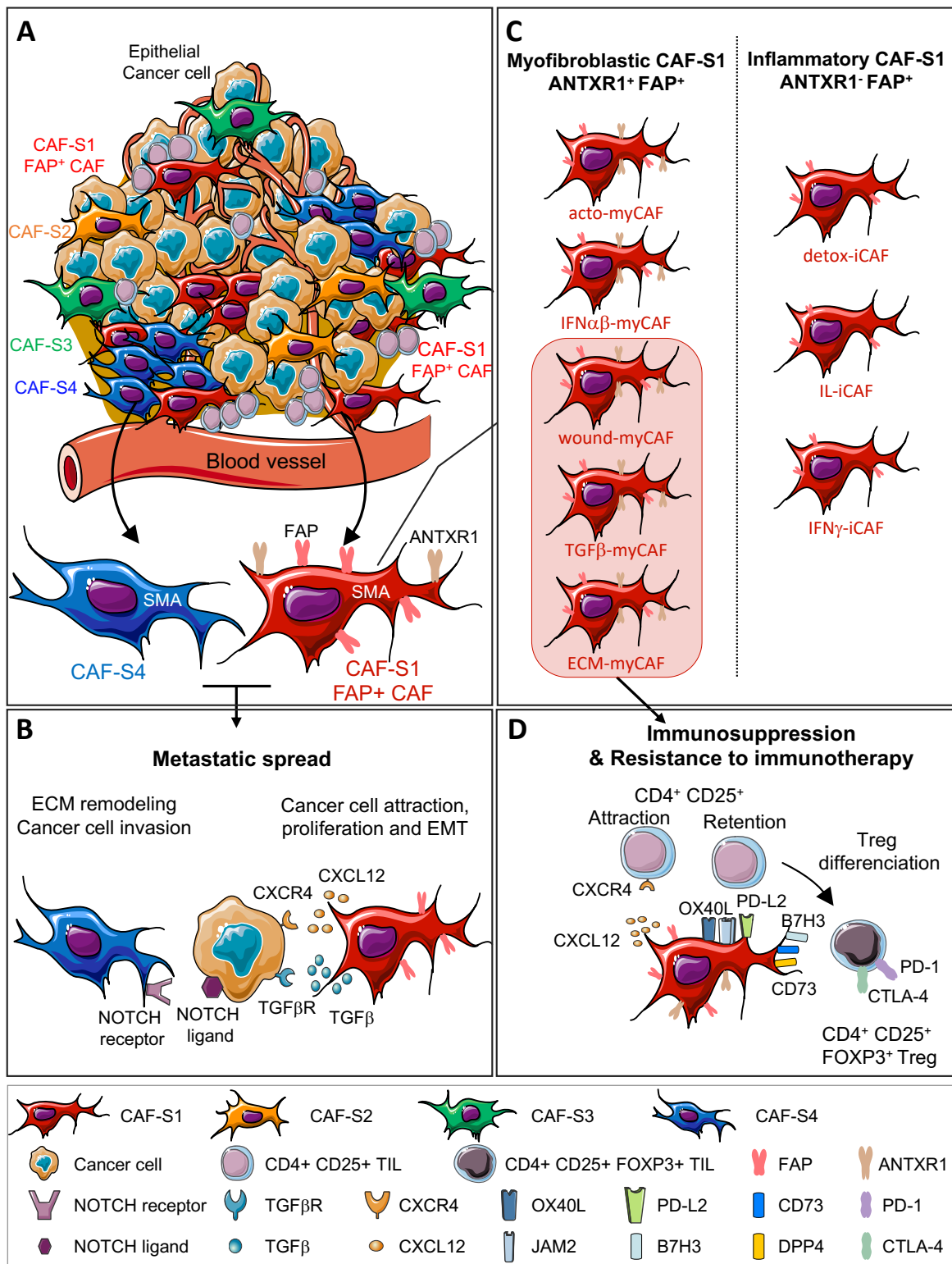


Figure 2: CAF heterogeneity and functions in cancer progression and resistance to immunotherapy

(A) Within the tumor microenvironment, 4 CAF subsets (CAF-S1 to CAF-S4) have been described. (B) CAF-S1 (FAP+) and CAF-S4 cooperate to induce metastatic spread by complementary mechanisms: CAF-S4 promote cancer cell invasion and ECM remodeling through the NOTCH signaling pathway and CAF-S1 induce cancer cell attraction, proliferation and epithelial-to-mesenchymal transition. (C) FAP+ CAF (CAF-S1) is a heterogeneous

population composed of 8 distinct cellular clusters: 5 myofibroblastic clusters (myCAF) and 3 inflammatory clusters (iCAF). myCAF and iCAF are distinguished by expression of the ANTXR1 membrane protein. (D) Among the myCAF (ANTXR1+), the ECM-myCAF, TGFb-myCAF and wound-myCAF clusters induce an immunosuppressive microenvironment enriched in regulatory T lymphocytes. They attract CD4+ CD25+ T cells through CXCL12, retain them by the action of OX40L, JAM2 and PD-L2 and induce their differentiation into CD4+ CD25+ FOXP3+ regulatory T lymphocytes through B7H3, CD73 and DPP4. These 3 CAF-S1 clusters are predictive of resistance to immunotherapy as they are enriched, at diagnosis, in non-responder patients treated with immune checkpoint inhibitors.

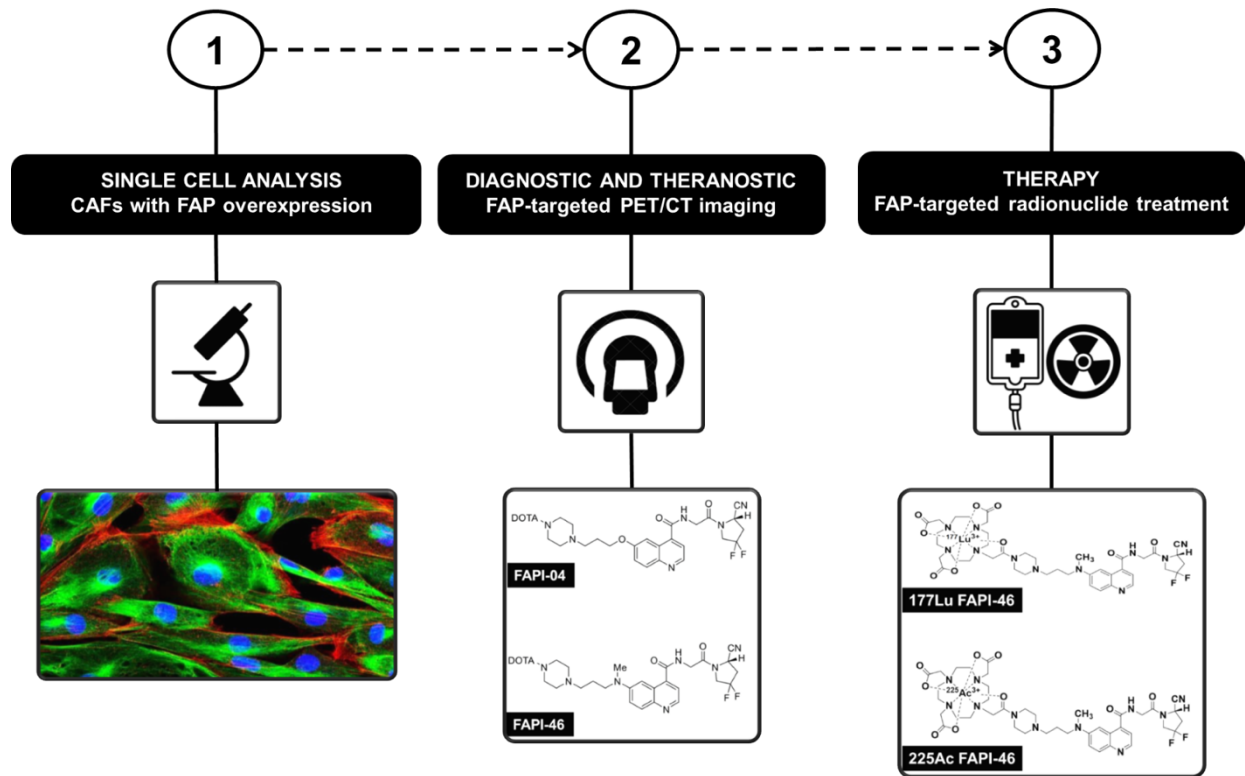


Figure 3: FAP, from basic research to clinical (diagnostic, theranostic and therapeutic) applications

Abbreviations: FAP (Fibroblast Activation Protein), CAFs (Cancer-Associated Fibroblasts), PET (Positron Emission Tomography), CT (Computed Tomography), FAPI (Fibroblast Activation Protein Inhibitor), ¹⁷⁷Lu (¹⁷⁷Lutetium), ²²⁵Ac (²²⁵Actinium).