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Pharmacologic Normalization of Pancreatic Cancer-Associated Fibroblast Secretome Impairs Prometastatic Cross-Talk With Macrophages

Rémi Samain,1 Alexia Brunel,1 Thibault Douché,1 Marjorie Fanjul,1 Stéphanie Cassant-Sourdy,1 Julia Rochotte,1 Jérôme Cros,2 Cindy Neuzillet,3 Jérôme Raffenne,1 Camille Duluc,1 Aurélie Perraud,4 Jérémy Nigri,5 Véronique Gigoux,1 Ivan Bieche,6 Matteo Ponzo,7 Gilles Carpentier,7 Ilaria Cascone,7 Richard Tomasini,5 Herbert A. Schmid,8 Muriel Mathonnet,4 Rémy Nicolle,9 Marie-Pierre Bousquet,10 Yvan Martineau,1 Stéphane Pyronnet,1 Christine Jean,1 and Corinne Bousquet1

1Centre de Recherches en Cancérologie de Toulouse (CRCT), Université de Toulouse, INSERM Unité Mixte de Recherche UMR-1037, CNRS Equipe de Recherche Labellisée ERL5294, Equipe de Recherche Labellisée “Ligue Contre le Cancer” & “LabEx Toucan”, Toulouse, France; 2Department of Pathology, Beaujon-Bichat University Hospital–Paris Diderot University, Clichy, France; 3Medical Oncology Department, Curie Institute, Versailles Saint-Quentin University, Saint Cloud, France; 4Equipe d’Accueil EA 3842 Laboratory, Medicine and Pharmacy Faculties, University of Limoges, Limoges, France; 5INSERM U1068/UMR 7258 CNRS, Cancer Research Center of Marseille, Marseille, France; 6Department of Genetics, Institut Curie, Paris Descartes University, Paris, France; 7Growth, Reparation and Tissue Regeneration Laboratory, Equipe de Recherche Labellisée ERL-CNRS 9215, University of Paris-Est, Créteil, France; 8Novartis Pharmaceuticals, Basel, Switzerland; 9Programme Cartes d’Identité des Tumeurs, Ligue Nationale Contre Le Cancer, Paris, France; 10Institute for Pharmacology and Structural Biology, University of Toulouse, Toulouse, France

SUMMARY
Cancer-associated fibroblasts orchestrate pancreatic adenocarcinoma (PDA) aggressiveness. We show that the Food and Drug Administration–approved somatostatin analog SOM230 inhibits metastasis in murine PDA models by acting through its receptor sst1 to disrupt the cancer-associated fibroblast production of colony stimulating factor 1, a prometastatic and macrophage-attracting chemokine whose stromal expression is associated with PDA aggressiveness.

BACKGROUND & AIMS: Cancer-associated fibroblasts (CAFs) from pancreatic adenocarcinoma (PDA) present high protein synthesis rates. CAFs express the G-protein–coupled somatostatin receptor sst1. The sst1 agonist SOM230 blocks CAF protumoral features in vitro and in immunocompromised mice. We have explored here the therapeutic potential of SOM230, and underlying mechanisms, in immunocompetent models of murine PDA mimicking the heavy fibrotic and immunosuppressive stroma observed in patient tumors.

METHODS: Large-scale mass spectrometry analyses were performed on media conditioned from 9 patient PDA-derived CAF cultures. Spontaneous transgenic and experimental (orthotopic co-graft of tumor cells plus CAFs) PDA-bearing mice were longitudinally ultrasound-monitored for tumor and metastatic progression. Histopathology and flow cytometry analyses were performed on primary tumors and metastases. Stromal signatures were functionally validated through bioinformatics using several published, and 1 original, PDA database.
**RESULTS:** Proteomics on the CAF secretome showed that SOM230 controls stromal activities including inflammatory responses. Among the identified secreted proteins, we validated that colony-stimulating factor 1 (CSF-1) (a macrophage growth factor) was reduced by SOM230 in the tumor and plasma of PDA-harboring mice, alongside intratumor stromal normalization (reduced CAF and macrophage activities), and dramatic metastasis reduction. In transgenic mice, these SOM230 benefits alleviate the chemotherapy-induced (gemcitabine) immunosuppressive stroma reshaping. Mechanistically, SOM230 acts in vivo on CAFs through sst1 to disrupt prometastatic CAF production of CSF-1 and cross-talk with macrophages. We found that in patients, stromal CSF-1 was associated with aggressive PDA forms.

**CONCLUSIONS:** We propose SOM230 as an antimetastatic therapy in PDA for its capacity to remodel the fibrotic and immunosuppressive myeloid stroma. This pharmacotherapy should benefit PDA patients treated with chemotherapies. (Cell Mol Gastroenterol Hepatol 2021;11:1405–1436; [https://doi.org/10.1016/j.jcmgh.2021.01.008])

**Keywords:** Antimetastatic Therapy; Cancer-Associated Fibroblasts; Pancreatic Adenocarcinoma; Stromal Cell Cross-Talk; Stroma Normalization; Macrophages; Somatostatin Receptor.

Pancreatic ductal adenocarcinoma (PDA) remains one of the deadliest neoplasms. Most patients die from metastatic disease within the first year after diagnosis. Stratifying PDA tumors using genetic profiles has not proven to be valuable for predicting therapeutic responses and clinical outcome in PDA, whereas transcriptomic profiles have defined 2 tumor subgroups in relation to tumor aggressiveness. Molecular profiles of the tumor and stromal compartments are reported to be of prognostic value. Consistently, a cross-talk between tumor cells and their stroma now is recognized as being critically involved in PDA progression and resistance to treatments. Hence, PDA tumors are characterized by a prominent desmoplastic reaction, which represents up to 80% of the tumor mass and comprises acellular organized matrix fibers produced mainly by activated fibroblasts within the tumor, termed cancer-associated fibroblasts (CAFs). CAFs are the most abundant cells in PDA stroma, and secrete, in addition to extracellular matrices, growth and angiogenic factors, as well as cytokines and chemokines, which favor tumor progression and chemoresistance. Immaturity of PDA stroma, linked with a high stromal cell content, correlates with a poor prognosis, emphasizing the critical role of stromal cells in dictating tumor cell behavior. In addition to CAFs, PDA stroma comprise immune cells, mostly tumor-associated macrophages (TAMs) of myeloid origin and polarized to an M2 phenotype with immunosuppressive activities. By secreting extracellular matrix remodeling enzymes; growth, angiogenic, and redox factors; and cytokines, this myeloid subset dampens antitumor immune reactions and facilitates tumor cell invasion and metastasis. In vitro studies also showed that pancreatic CAFs induce monocyte polarization into immunosuppressive M2-like macrophages, suggesting that a dynamic cross-talk exists between these stromal cells, impacting tumor cell biology. Thus, PDA stroma plays a prominent role in its aggressiveness and resistance to therapy, yet remains difficult to target owing to its cellular and functional heterogeneity. The use of murine models mimicking the complex interactions between pancreatic cancer cells and their microenvironment offers research opportunities in a therapeutic perspective. Using in vitro settings and an immunocompromised mouse model of PDA, our previous work has shown that protein synthesis dependent on the Akt-mechanistic Target Of Rapamycin (mTOR) pathway is abnormally activated in primary cultures of CAFs compared with nonactivated pancreatic stellate cells (PSCs) (isolated from healthy pancreas), and is required for the promotion of pancreatic tumor cell metastasis and chemoprotection. Hence, pharmacologic inhibition of this pathway in CAFs abrogates the translation of messenger RNA (mRNA) encoding the secreted protein interleukin 6, directly impacting tumor cell epithelial-to-mesenchymal transition and chemoresistance. Inhibition of the Akt-mTOR pathway was achieved using the somatostatin analog SOM230 (Pasireotide; Novartis, Basel, Switzerland) targeting the somatostatin G-protein–coupled receptor subtype 1 (GPCR) sst1, which we identified to be expressed in CAFs but not in PSCs, as later confirmed by others.

Here, we aimed at exhaustively uncovering the impact of SOM230 on CAFs to identify mechanisms for its putative therapeutic benefit in relevant immunocompetent murine models of experimental and spontaneous PDA. Large-scale, mass-spectrometry (MS) analyses of the secretome of 9 patient tumor-derived CAF primary cultures showed SOM230-inhibited proteins enriched in published PDA “stroma-activated” signatures, and involved in inflammatory responses. Accordingly, SOM230 showed antimetastatic activity in relevant PDA-harboring mice, normalizing the fibrotic and immunosuppressive myeloid stroma, including the cytokinin storm reaction induced by chemotherapy (gemcitabine). Mechanistically, SOM230 acts through the CAF-expressed sst1 receptor to disrupt a prometastatic cross-talk between CAFs and immunosuppressive TAMs involving the chemokine colony-stimulating factor (CSF-1).
whose specific expression in the stroma of patient tumors was found to be associated with aggressive and metastatic forms of PDA.

**Results**

**SOM230 Decreases CAF Production of Secreted Proteins Enriched in PDA Stroma-Activated Signatures**

To exhaustively identify CAF-secreted proteins, we performed MS analyses on media conditioned by 9 CAF explants, treated or not with SOM230 for 48 hours (Figure 1A). A total of 272 proteins were identified as being significantly ($P < .05$) down-regulated (threshold of 2-fold) by SOM230, but none were up-regulated (Figure 1B, Table 1). These proteins are graphically represented for each CAF in heatmaps with box plots for global levels (Figure 1C), and averaged in the secretomes of the 9 CAF cultures (Figure 1D) where pairs between SOM230-treated and control untreated conditions are indicated. Gene set enrichment analysis (GSEA) for this SOM230-regulated secretomic signature identified pathways including Phosphoinositide 3-kinase (PI3K-AKT) and mTOR signalings and the inflammatory response pathway (Figure 1E). Among the 272 identified proteins, 75 and 54 were common to the “stroma-activated” signature from Moffitt et al., or from Puleo et al., respectively (Figure 1F), with a significant normalized enrichment score (Figure 1G), and 47 proteins were common to both signatures (listed in Figure 1H). Dependency on sst1 expression of several of the identified SOM230-regulated secretomic signatures (biglycan, connective tissue growth factor, and thrombospondin 2 in Table 1) was confirmed in human CAFs knocked-down for sst1 (Figure 1I). These results suggest that SOM230 normalizes numerous cross-talks originating from sst1-expressing CAFs, putatively regulating both tumor cells and stromal biology.

**SOM230 Reduces Metastasis in PDA Mouse Models Through the GPCR sst1 Expressed on CAFs**

We previously showed that SOM230 is antimetastatic in immunocompromised mice, by indirectly, through its action on CAFs, inhibiting epithelial-to-mesenchymal transition in PDA tumor cells. To evaluate whether SOM230 normalized the PDA stroma in models closer to the clinic, we developed an immunocompetent murine model consisting of the orthotopic co-graft of syngeneic C57BL/6 tumor cells (derived from Pdx-1-Cre; LSL-KrasG12D/; LSL-Trp53R172H/+ tumors [KPC tumors]), with PSCs isolated from either wild-type (WT) or sst1 knock-out (KOsst1) C57BL/6 mouse pancreata (Figure 2A and B). First, these PSCs were characterized phenotypically. Ex vivo–grown WT mice PSCs were activated (as attested by up-regulation of α smooth muscle actin [αSMA] expression) and spontaneously acquired the expression of GPCR sst1 (Figure 2C). They also presented high protein synthesis rates (monitored by surface sensing of translation [SU NabSET]). As we previously published in human CAFs, these rates were reduced by SOM230, as was the expression of the extracellular matrix (ECM) protein and CAF marker perioscin (Figure 2D). Accordingly, perioscin was identified in the SOM230-regulated, patient-derived CAF secretomic signature (Table 1). KOsst1 mice PSCs showed equivalent high intrinsic protein synthesis rates and perioscin expression as WT PSCs, but were not responsive to SOM230 treatment (Figure 2D). WT or KOsst1 PSCs then were co-grafted with tumor cells in C57BL/6 mice, which were later treated or not with SOM230 once tumors were formed, as indicated (Figure 2E). No SOM230 effect was observed on primary tumor growth in any group (Figure 2F), whereas the drug significantly reduced lung metastasis incidence and tumor load (defined as the cumulative lung metastatic area, less than or more than 0.1% of the total lung area) in mice co-grafted with tumor cells and WT PSCs (Figure 2G and H). No liver metastases were observed in this model. SOM230 did not significantly inhibit metastasis in mice co-grafted with KOsst1 PSCs. Altogether, these results show that SOM230 is antimetastatic in a novel immunocompetent PDA model because of its action on sst1 expressed by CAFs.

**SOM230 Inhibits Metastasis by Decreasing CAF-Secreted CSF-1**

To identify CAF-secreted proteins involved in the drug antimetastatic effect, we searched for factors down-regulated by SOM230 both in the CAF secretome (Table 1) and in the plasma of mice grafted with KPC tumor cells plus PSCs and treated with SOM230 (from Figure 2) (Figure 3A and B, Table 2). Five common proteins were identified (Figure 3C), including perioscin and the chemokine CSF-1. We focused on CAF-secreted CSF-1. We confirmed by enzyme-linked immunosorbent assay (ELISA) that SOM230 decreased CSF-1 in patient CAF-conditioned media (Figure 3D). Importantly, we found that sst1-expressing CAFs also co-expressed CSF-1, as assessed by confocal immunofluorescence imaging of patient PDA tissues (Figure 3E). In the plasma of grafted and SOM230-treated mice (from Figure 2), we confirmed that SOM230 decreased the CSF-1 concentration in a dependent manner to CAF-expressed sst1 (Figure 3F). CSF-1 expression also was reduced by SOM230 in the primary tumors of treated mice (Figure 3G) (the different 50- to 60-kilodalton-weight bands visualized by Western blot are consistent with the described spliced and matured forms of CSF-1). To test the role of CAF-secreted CSF-1 on the antimetastatic effect of SOM230, KPC tumor cells then were co-grafted in the pancreas of syngeneic mice with WT or CSF-1–overproducing and secreting PSCs (CSF-1 PSCs). We confirmed by ELISA a high CSF-1 production in conditioned media of CSF-1 PSCs (122 ± 5 ng/mL), which SOM230 was unable to reduce (Figure 3H). Upon orthotopic co-graft in C57BL/6 mice of tumor cells with WT or CSF-1 PSCs, no significant difference in tumor growth or in metastatic potential was observed in the untreated condition, and SOM230 did not impact primary tumor growth in WT PSCs or in CSF-1–transduced PSCs (Figure 3I and J). However, the
A. CAFs ± SOM230, 48h

Conditioned media

2.5% (w/w) of heavy-labeled proteins

Denaturation
SDS-PAGE

Digestion

Peptide extraction

LC-MS/MS

B. Fold-change 2

log10 FC SOM230 / NT

C. Pathway Gene ranks NES P-value

| Pathway                                      | Gene ranks | NES  | P-value |
|----------------------------------------------|------------|------|---------|
| Focal adhesion (KEGG)                        |            | 1.40 | 8.5e-03 |
| Hippo signaling pathway (KEGG)                |            | 1.54 | 5.1e-03 |
| Inflammatory Response Pathway (WikiPathway)  |            | 1.58 | 4.6e-03 |
| Leukocyte transendothelial migration (KEGG)  |            | 1.65 | 1.5e-03 |
| PI3K-Akt signaling (KEGG)                     |            | 1.45 | 2.0e-03 |
| α6β1 α6β4 integrin (PID)                      |            | 1.68 | 3.1e-04 |
| α6β1 integrin (PID)                           |            | 1.61 | 1.7e-03 |
| Nuclear Beta Catenin (PID)                    |            | 1.59 | 1.7e-03 |
| Axon guidance (Reactome)                      |            | 1.47 | 7.0e-04 |
| mTOR pathway (PID)                            |            | 1.64 | 1.3e-03 |
| Glycosaminoglycan degradation (KEGG)          |            | 1.63 | 7.5e-04 |

D. Average protein quantification (log2)

E. Activated stroma genes

F. Secretome

Moffit_Activated stroma

- NES = 1.4
  - P-value = 1e-04

Puelo_Activated stroma

- NES = 1.4
  - P-value = 4e-04

G. Activated stroma_Moffit et al, Gene set

H. Activated stroma_Puelo et al, Gene set

CAFs ± SOM230, 48h

Biglycan (nmol of 10^6 cells)

CTGF (mg/mL of 10^6 cells)

TIMP2 (ng/mL of 10^6 cells)

CAF siCTR

CAF siSst1

CAF siCTR

CAF siSst1

CAF siCTR

CAF siSst1

CAF siCTR

CAF siSst1

GAPDH

CAF

GAPDH
expected SOM230 inhibitory effect on lung metastasis incidence and load observed with WT PSCs was abrogated when PSCs overexpressed secreted CSF-1 (Figure 3J and K). These results show the critical role of CAF-derived CSF-1 in the antimetastatic effect of SOM230.

SOM230, Acting Through the CAF-Expressed GPCR sst1, Normalizes the Inflammatory PDA Stroma

Because the CSF-1 chemokine is involved in monocyte recruitment and activation, we performed fluorescence-activated cell sorter analysis on pancreatic tumors grown in mice co-grafted with tumor cells plus WT or KO st1 PSCs (Figure 4A and B). SOM230 treatment did not significantly impact intratumor load of total CD45, but decreased that of activated stroma signatures from the Mof used to generate SOM230-treated and untreated conditions are indicated by a gray segment. The Wilcoxon matched-pairs signed rank test was performed (Figure 4C–F). In contrast, no significant effect of SOM230 on macrophage subsets was observed when KO st1 PSCs were co-grafted (Figure 4D–F). SOM230 treatment did not impact the proportions of CD3+ , CD4+ , and CD8+ lymphoid cell populations (Figure 4G–I). These results indicate that SOM230 decreases the intratumor macrophage load, including the F4/80+CD11b+CD206+ subset comprising TAMs.

SOM230 Is Antimetastatic in a Murine Spontaneous Model of PDA

The therapeutic and stroma-normalizing benefits of SOM230 were tested in KPC mice, developing a spontaneous PDA recognized to closely mimic the human pathology and its fibrotic immune stroma. We showed by immunohistochemistry that sst1 was expressed in the αSMA-positive KPC tumor stroma (Figure 5A). Because all PDA patients receive chemotherapy and to get closest to clinical reality, SOM230 treatment of KPC mice also was evaluated in a chemotherapy background (gemcitabine), as indicated (Figure 5B), starting at equal ultrasound-calculated mean volumes between treatment groups (Figure 5C). We observed that tumor volume progression, which was exponential from day 1 to 21 in untreated mice, was reduced significantly in the combination-treated group (gemcitabine plus SOM230), but not by gemcitabine or SOM230 alone (Figure 5D–H). The combination therapy also significantly reduced tumor growth and extended survival, compared with untreated or gemcitabine-treated mice, in another spontaneous transgenic mouse PDA model (Pdx1-Cre; LSL-KrasG12D; Ink4a/fl/fl), in which gemcitabine alone also was efficient (Figure 5I). In KPC mice, metastatic incidence and load were analyzed in the liver (only rare lung micrometric metastases were visualized). This was comparatively quantified in KPC mice presenting equivalent primary tumor volume after killing (Table 3), to avoid the evaluation of a drug antimetastatic potential directly linked to its delaying effect on primary tumor growth. The combination therapy significantly reduced liver metastatic incidence and load because only 4 of 8 mice in this group presented liver metastases with a low metastatic load (defined as <3% of cumulative liver metastatic area), against 7 of 8 in the untreated or gemcitabine-treated group, which presented in most cases a high metastatic load (Figure 5K and L). In contrast, SOM230 did not have an antimetastatic effect when administered alone, although 2 of 8 SOM230-treated mice, whose tumor growth was delayed by SOM230 (mice number 734 and 1027) (Figure 5G, Table 3), also were nonmetastatic. Only the combination therapy significantly extended mouse survival, as compared with no treatment (Figure 5M). These results show that SOM230 is inefficient to inhibit metastatic spread in KPC mice when used alone, but is antimetastatic in a gemcitabine-treated background.

Immunopathology of KPC primary tumors showed that SOM230 treatment, when combined with chemotherapy, did not increase the cytotoxicity of gemcitabine, which...
| Protein names                          | Gene names | Moy ratio NT/SOM230 | P value     |
|---------------------------------------|------------|---------------------|-------------|
| Decorin                               | DCN        | 3565.20             | .0037699    |
| Biglycan                              | BGN        | 2416.98             | .0105944    |
| Thrombospondin-2                      | THBS2      | 993.52              | .0002836    |
| Glia-derived nexin                    | SERPINE2   | 944.33              | .0002733    |
| Adipocyte enhancer-binding protein 1  | AEBP1      | 629.64              | .0053412    |
| Agrin                                 | AGRN       | 618.31              | .0478032    |
| Fibulin-1                             | FBLN1      | 572.34              | .0065804    |
| Peroxidasin homolog                   | PXDN       | 468.76              | .0481937    |
| Carboxypeptidase E                    | CPE        | 448.97              | .0017332    |
| HLA class I histocompatibility antigen| HLA-A      | 412.95              | .0003536    |
| Protein-lysine 6-oxidase              | LOX        | 405.09              | .0007965    |
| Elongation factor 1-δ                 | EEF1D      | 401.99              | .0015514    |
| Stromelysin-1                         | MMP3       | 369.77              | .0387783    |
| SPARC                                 | SPARC      | 334.84              | .0028440    |
| Glyceraldehyde-3-phosphate dehydrogenase, testis-specific | GAPDH | 302.95 | .0124452 |
| Coiled-coil domain-containing protein 80 | CCDC80 | 269.82 | .0124452 |
| Calumenin                             | CALU       | 244.86              | .0154646    |
| Out at first protein homolog          | OAF        | 232.27              | .0072591    |
| Fibrillin-1                           | FBN1       | 223.43              | .0085725    |
| Testican-1                            | SPOCK1     | 202.29              | .0014202    |
| Connective tissue growth factor       | CTGF       | 189.63              | .0180345    |
| Fibulin-2                             | FBLN2      | 160.13              | .0030805    |
| Collagen α-1(V) chain                 | COL5A1     | 153.20              | .0376581    |
| Syndecan-4                            | SDC4       | 142.77              | .0181431    |
| Phospholipid transfer protein         | PLTP       | 140.32              | .0027074    |
| A disintegrin and metalloproteinase with thrombospondin motifs 1 | ADAMTS1 | 139.38 | .0020898 |
| Sushi repeat-containing protein SRPX2 | SRPX2      | 131.96              | .0014868    |
| Complement C1r subcomponent           | C1R        | 129.50              | .0073546    |
| Sushi repeat-containing protein SRPX   | SRPX       | 116.75              | .0239367    |
| Vitamin K-dependent protein S         | PROS1      | 115.14              | .0483223    |
| Actin, cytoplasmic 1; actin, cytoplasmic 1, N-terminally processed | ACTB | 112.36 | .0005106 |
| Plasma α-L-fucosidase                 | FUC2       | 108.05              | .0004376    |
| Laminin subunit γ-1                   | LAMC1      | 97.78               | .0000042    |
| Inhibin α A chain                     | INHBA      | 93.39               | .0471213    |
| Proliferating cell nuclear antigen    | PCNA       | 89.42               | .0306750    |
| Stanniocalcin-2                       | STC2       | 84.81               | .018231     |
| Tumor necrosis factor–receptor superfamily member 11B | TNFRSF11B | 82.61 | .0418658 |
| Tryptophan–transfer RNA ligase         | WARS       | 79.58               | .0028410    |
| Latent-transforming growth factor β-binding protein 1 | LTBP1 | 78.27 | .0084952 |
| Myosin-14                             | MYH14      | 76.43               | .0017097    |
| Gelsolin                              | GSN        | 76.16               | .0128688    |
| Latent-transforming growth factor β-binding protein 2 | LTBP2 | 74.59 | .0008218 |
| Neutrín-4                             | NTN4       | 67.79               | .026525     |
| Protein transport protein Sec23A       | SEC23A     | 65.55               | .0023638    |
| Coagulation factor IX                 | F9         | 64.96               | .0289241    |
| Septin-7                              | SEPT-7     | 64.92               | .0419335    |
| Microfibrillar-associated protein 2    | MFAP2      | 64.75               | .0011975    |
| Collagen α-2(I) chain                 | COL1A2     | 64.69               | .0013190    |
| Serine protease HTRA1                  | HTRA1      | 62.57               | .0039875    |
| Protein names | Gene names | Moy ratio NT/SOM230 | P value |
|---------------|------------|---------------------|---------|
| Collagen α-1(III) chain | COL3A1 | 57.28 | .0010022 |
| γ-enolase | ENO2 | 56.15 | .0059011 |
| Tripeptidyl-peptidase 1 | TPP1 | 53.61 | .0135799 |
| β-hexosaminidase subunit β | HEXB | 51.51 | .0000029 |
| T-complex protein 1 subunit γ | CCT3 | 51.00 | .0118711 |
| Amyloid-like protein 2 | APLP2 | 48.18 | .0001436 |
| Urotensin-2 | UTS2 | 47.26 | .0307781 |
| 60S acidic ribosomal protein P1 | RPLP1 | 45.66 | .0020590 |
| Angiopoietin-related protein 2 | ANGPTL2 | 43.54 | .0396281 |
| Nascent polypeptide-associated complex subunit α | NACA | 43.35 | .0397817 |
| Complement C1s subcomponent | C1S | 43.01 | .0212752 |
| Glutathione S-transferase P | GSTP1 | 42.75 | .005148 |
| Calsyntenin-1; soluble Alc-α; CTF1-α | CLSTN1 | 41.57 | .0000025 |
| Ras-related protein Rab-6A | RAB6A | 38.84 | .0354047 |
| Proactivator polypeptide; saposin-A | PSAP | 37.12 | .000017 |
| Dickkopf-related protein 3 | DKK3 | 37.04 | .0064965 |
| F-actin-capping protein subunit β | CAPZB | 36.85 | .006800 |
| Tubulin α-4A chain | TUBA4A | 36.76 | .029517 |
| Clathrin heavy chain 1 | CLTC | 36.73 | .0173279 |
| Deoxyuridine 5-triphosphate nucleotidohydrolase, mitochondrial | DUT | 36.34 | .0021730 |
| Proteasome subunit β type-6 | PSMB6 | 35.95 | .0444021 |
| Proprotein convertase subtilisin/kexin type 9 | PCSK9 | 35.87 | .0432914 |
| 72-kilodalton type IV collagenase; PEX | MMP2 | 35.79 | .0004569 |
| Prolyl 3-hydroxylase 1 | LEPRE1 | 35.26 | .0496856 |
| Lactadherin; lactadherin short form; medin | MFGE8 | 32.58 | .003940 |
| Elongation factor 1-γ | EIF1G | 31.24 | .0469751 |
| Ferritin light chain | FTL | 29.77 | .0450262 |
| Lupus La protein | SSB | 29.38 | .002821 |
| Follistatin-related protein 5 | FSTL5 | 29.37 | .034505 |
| Matrix-remodeling-associated protein 5 | MXRA5 | 27.17 | .0056534 |
| Eukaryotic initiation factor 4A-II | EIF4A2 | 27.13 | .0271990 |
| WD repeat-containing protein 65 | WDR65 | 27.05 | .0052157 |
| Extracellular sulfatase Sulf-1 | SULF1 | 26.38 | .0003432 |
| Calponin-3 | CNN3 | 26.17 | .014325 |
| Protein phosphatase 2 scaffold subunit α | PPP2R1A | 25.84 | .0446915 |
| β-1,4-galactosyltransferase 5 | B4GALT5 | 25.78 | .0196639 |
| Serum albumin | ALB | 25.68 | .0000003 |
| Synaptic vesicle membrane protein VAT-1 homolog | VAT1 | 25.46 | .000010 |
| Splicing factor, proline- and glutamine-rich | SFPO | 24.92 | .0379895 |
| Glucose-6-phosphate 1-dehydrogenase | G6PD | 24.56 | .0146303 |
| Cochlcin | COCH | 24.14 | .038765 |
| Platelet-activating factor acetylhydrolase IB subunit α | PAFAH1B1 | 23.88 | .003566 |
| Extracellular matrix protein 1 | ECM1 | 23.79 | .0160787 |
| Vacular protein sorting-associated protein 35 | VPS35 | 23.12 | .0179969 |
| Exostosin-1 | EXT1 | 22.83 | .0080334 |
| Protein S100-A6 | S100A6 | 21.49 | .0368965 |
| Neutral α-glucosidase AB | GANAB | 21.27 | .001394 |
| Podocan | PODN | 20.85 | .020030 |
| Fascin | FSCN1 | 20.41 | .002658 |
| Protein names | Gene names | Moy ratio NT/SOM230 | P value |
|---------------|------------|---------------------|---------|
| Olfactomedin-like protein 3 | OLFML3 | 19.10 | .0087429 |
| T-complex protein 1 subunit η | CCT7 | 19.08 | .0349927 |
| Membrane-bound transcription factor site-1 protease | MBTPS1 | 18.28 | .0444638 |
| Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3 | PLOD3 | 18.25 | .0290330 |
| Heterogeneous nuclear ribonucleoprotein K | HNRNPK | 18.10 | .0055013 |
| Peroxiredoxin-4 | PRDX4 | 17.52 | .0163462 |
| Heat shock protein 75 kilodaltons, mitochondrial | TRAP1 | 16.80 | .0203181 |
| ANK repeat and PH domain-containing protein 1 | ACAP1 | 15.73 | .0469891 |
| Clusterin; clusterin β chain; clusterin α chain | CLU | 15.19 | .0000107 |
| Macrophage colony-stimulating factor 1 | CSF1 | 15.06 | .0234921 |
| EMILIN-1 | EMILIN1 | 14.74 | .0335435 |
| Low-density lipoprotein receptor | LDLR | 14.45 | .0122306 |
| Laminin subunit β-2 | LAMB2 | 14.41 | .0025413 |
| Carboxyl reductase [reduced nicotinamide adenine dinucleotide phosphate] 3 | CBR3 | 14.29 | .0040576 |
| Serpin B6 | SERPINB6 | 13.24 | .0184994 |
| 60-kilodalton heat shock protein, mitochondrial | HSPD1 | 12.71 | .0000572 |
| Adenosine triphosphate-citrate synthase | ACLY | 12.46 | .0495593 |
| Glypican-1; secreted glypican-1 | GPC1 | 11.88 | .0054106 |
| Periostin | POSTN | 11.34 | .0000412 |
| 45-kilodalton calcium-binding protein | SDF4 | 11.31 | .0000878 |
| Importin-5 | IPOS | 11.24 | .0232329 |
| Inositol monophosphatase 1 | IMPA1 | 11.10 | .0017802 |
| Fibulin-5 | FBLN5 | 11.09 | .0004997 |
| Collagen triple-helix repeat-containing protein 1 | CTHRC1 | 10.55 | .0246140 |
| Glutathione S-transferase Ω-1 | GSTO1 | 10.24 | .0134160 |
| β-2-microglobulin | B2M | 9.94 | .0032569 |
| Actin-related protein 3 | ACTR3 | 9.67 | .0009390 |
| Transcriptional activator protein Pur-β | PURB | 9.53 | .0046924 |
| Elongation factor 1-β | EEF1B2 | 9.41 | .0125778 |
| Protein phosphatase 1 regulatory subunit 12A | PPP1R12A | 9.11 | .0201546 |
| Nucleobindin-2 | NUCB2 | 8.99 | .0004586 |
| Fibronectin | FN1 | 8.92 | .0001491 |
| Collagen α-2(VI) chain | COL6A2 | 8.87 | .0018644 |
| 6-phosphogluconate dehydrogenase, decarboxylating | PGD | 8.79 | .0040032 |
| Amyloid β A4 protein | APP | 8.77 | .0005750 |
| Transferrin-receptor protein 1 | TFRC | 8.65 | .0001086 |
| Annexin A2 | ANXA2 | 8.64 | .0301342 |
| Elongation factor 2 | EEF2 | 8.11 | .0000368 |
| Acetyl-CoA acetyltransferase, cytosolic | ACAT2 | 8.05 | .0340549 |
| α-soluble NSF (attachment protein | NAPA | 7.90 | .0118155 |
| Epidermal growth factor-containing fibulin-like extracellular matrix protein 2 | EFEMP2 | 7.84 | .0003193 |
| Heat shock 70-kilodalton protein 1A/1B | HSPA1A | 7.64 | .0088805 |
| Ubiquitin-like modifier-activating enzyme 1 | UBA1 | 7.44 | .0052055 |
| Talin-1 | TLN1 | 7.41 | .0000026 |
| Tropomyosin α-3 chain | TPM3 | 7.41 | .0022479 |
| Nucleophosmin | NPM1 | 7.14 | .0005244 |
| Thrombospondin-1 | THBS1 | 6.59 | .0086199 |
| β-hexosaminidase subunit α | HEXA | 6.47 | .0019689 |
| Protein names                                                                 | Gene names     | Moy ratio NT/SOM230 | P value |
|-----------------------------------------------------------------------------|----------------|---------------------|---------|
| Cysteine–transfer RNA ligase, cytoplasmic                                    | CARS           | 6.47                | 0.0033002 |
| C-type lectin domain family 11 member A                                     | CLEC11A        | 6.34                | 0.002634 |
| Cysteine and glycine-rich protein 1                                          | CSRP1          | 6.23                | 0.0157997 |
| Galectin-3-binding protein                                                   | LGALS3BP       | 6.20                | 0.003411 |
| Nucleobindin-1                                                               | NUCB1          | 6.19                | 0.000158 |
| Proteasome subunit β type-4                                                  | PSMB4          | 5.86                | 0.0014785 |
| Follistatin-related protein 1                                                | FSTL1          | 5.86                | 0.0002589 |
| Ras guanosine triphosphatase-activating-like protein IQGAP1 (IQ Motif Containing GTPase Activating Protein 1) | IQGAP1         | 5.68                | 0.0108885 |
| Ribonuclease inhibitor                                                       | RNH1           | 5.63                | 0.0057533 |
| Actin, α cardiac muscle 1                                                    | ACTC1          | 5.55                | 0.002417 |
| Cathepsin B                                                                  | CTSB           | 5.53                | 0.0145813 |
| Protein disulfide-isomerase A4                                               | PDIA4          | 5.51                | 0.0109743 |
| Myosin light polypeptide 6                                                   | MYL6           | 5.45                | 0.003450 |
| Serine/threonine-protein phosphatase 1 (PP1)–β catalytic subunit            | PPP1CB         | 5.36                | 0.0194514 |
| Hsp90 co-chaperone Cdc37                                                     | CDC37          | 5.34                | 0.0088745 |
| Transforming growth factor-β–induced protein ig-h3                          | TGFBI          | 5.33                | 0.004158 |
| Purine nucleoside phosphorylase                                              | PNP            | 5.26                | 0.0437861 |
| Collagen α-1(XII) chain                                                      | COL12A1        | 5.22                | 0.030738 |
| T-complex protein 1 subunit β                                                | CCT8           | 5.14                | 0.0419163 |
| Peroxiredoxin-2                                                             | PRDX2          | 5.03                | 0.0113992 |
| Epidermal growth factor-containing fibulin-like extracellular matrix protein 1 | EFEMP1         | 5.00                | 0.000249 |
| Nucleosome assembly protein 1-like 4                                          | NAP1L4         | 4.88                | 0.008493 |
| Myosin-9                                                                    | MYH9           | 4.88                | 0.021392 |
| Tubulin β chain                                                              | TUBB           | 4.85                | 0.006886 |
| Hsc70-interacting protein                                                    | ST13           | 4.81                | 0.0047255 |
| Importin subunit α-3                                                         | KPNAA4         | 4.81                | 0.0059251 |
| Sulphydryl oxidase 1                                                         | QSOX1          | 4.74                | 0.0163887 |
| Tubulin β-4B chain                                                           | TUBB4B         | 4.68                | 0.000303 |
| Nidogen-1                                                                   | NID1           | 4.58                | 0.0176328 |
| Cathepsin D                                                                 | CTSD           | 4.57                | 0.000334 |
| Adenyl cyclase-associated protein 1                                           | CAP1           | 4.51                | 0.000022 |
| Importin subunit β-1                                                         | KPNB1          | 4.51                | 0.011822 |
| Laminin subunit β-1                                                          | LAMB1          | 4.46                | 0.016507 |
| WD repeat-containing protein 1                                                | WDR1           | 4.45                | 0.000318 |
| Tubulin α-1B chain                                                           | TUBA1B         | 4.43                | 0.000209 |
| Aspartate aminotransferase, mitochondrial                                    | GOT2           | 4.42                | 0.004496 |
| Proteasome subunit α type-6                                                  | PSMA6          | 4.39                | 0.0317503 |
| Heat shock protein HSP 90–α                                                   | HSP90AA1       | 4.33                | 0.000011 |
| Coronin-1C                                                                  | CORO1C         | 4.26                | 0.000013 |
| Eukaryotic initiation factor 4A-I                                            | EIF4A1         | 4.24                | 0.0059134 |
| Transgelin-2                                                                 | TAGLN2         | 4.20                | 0.0022821 |
| Heat shock cognate 71-kilodalton protein                                     | HSPA8          | 4.20                | 0.0125521 |
| Nucleoside diphosphate kinase B                                              | NME2           | 4.15                | 0.0049568 |
| Actin, cytoplasmic 2                                                          | ACTG1          | 4.08                | 0.000034 |
| Neuruplin-2                                                                  | NRP2           | 4.06                | 0.0175685 |
| Pentraxin-related protein PTX3                                                | PTX3           | 4.04                | 0.0007182 |
| Nucleolin                                                                   | NCL            | 4.01                | 0.0276738 |
| Filamin-A                                                                    | FLNA           | 4.01                | 0.0003733 |
Table 1. Continued

| Protein names                                | Gene names | Moy ratio NT/SOM230 | P value  |
|----------------------------------------------|------------|---------------------|----------|
| Fibromodulin                                 | FMOD       | 3.98                | .0092232 |
| Plasminogen activator inhibitor 1            | SERPINE1   | 3.97                | .0008984 |
| Chloride intracellular channel protein 4     | CLIC4      | 3.92                | .0008258 |
| Multiple inositol polyphosphate phosphatase 1| MINPP1     | 3.91                | .0418345 |
| Neurofilament-1                              | NRP1       | 3.91                | .0167928 |
| Heat shock protein HSP 90-β                  | HSP90AB1   | 3.91                | .0000448 |
| 14-3-3 protein Σ                            | SFN        | 3.88                | .010093  |
| Heterogeneous nuclear ribonucleoprotein D0   | HNRNPD     | 3.84                | .0028596 |
| Reticulocalbin-1                            | RCN1       | 3.83                | .001862  |
| Vimentin                                     | VIM        | 3.81                | .0081098 |
| Putative elongation factor 1-α-like 3        | EEF1A1P5   | 3.80                | .0005683 |
| Lumican                                      | LUM        | 3.80                | .0000514 |
| Exostosin-2                                  | EXT2       | 3.79                | .0233352 |
| Puromycin-sensitive aminopeptidase            | NPEPPS     | 3.79                | .0045265 |
| Glutaminyl-peptide cyclotransferase          | QPCT       | 3.76                | .0074783 |
| Pyruvate kinase PKM                          | PKM        | 3.76                | .0006099 |
| C-Jun-amino-terminal kinase-interacting protein 4 | SPAG9     | 3.74                | .0003581 |
| Poly(rC)-binding protein 1                   | PCBP1      | 3.71                | .0004751 |
| Farnesyl pyrophosphate synthase              | FDPS       | 3.55                | .0033517 |
| Tropomyosin α-4 chain                        | TPM4       | 3.53                | .0022587 |
| Transitional endoplasmic reticulum adenosine triphosphatase | VCP     | 3.52                | .0000575 |
| Hyaluronan-binding protein 2                 | HABP2      | 3.50                | .0021050 |
| Serglycin                                    | SRGN       | 3.49                | .041091  |
| Metalloproteinase inhibitor 2                | TIMP2      | 3.46                | .0000760 |
| Phosphoglucomutase-1                         | PGM1       | 3.43                | .0083578 |
| Filamin-C                                    | FLNC       | 3.42                | .0073859 |
| Heat shock 70-kilodalton protein 6            | HSPA6      | 3.42                | .0002413 |
| 78-kilodalton glucose-regulated protein       | HSPA5      | 3.36                | .0026891 |
| Dihydropyrimidinase-related protein 2        | DPYSL2     | 3.36                | .0000008 |
| Protein CYR61                                 | CYR61      | 3.33                | .0021073 |
| Chloride intracellular channel protein 1      | CLIC1      | 3.32                | .0000008 |
| α-actinin-1                                  | ACTN1      | 3.29                | .0025329 |
| Histone H2A type 2-C; histone H2A type 2-A   | HIST2H2AC  | 3.29                | .0061036 |
| Ubiquitin carboxyl-terminal hydrolase isozyme L1 | UCHL1     | 3.28                | .0423517 |
| Proteasome subunit α type-1                  | PSMA1      | 3.20                | .0008972 |
| Ras-related protein Rap-1A                   | RAP1A      | 3.19                | .0000007 |
| Gremlin-1                                    | GREM1      | 3.18                | .0497429 |
| γ-glutamyl hydrolase                         | GGH        | 3.17                | .0003584 |
| 14-3-3 protein γ                             | YWHAH      | 3.17                | .0004627 |
| Peroxiredoxin-1                              | PRDX1      | 3.14                | .000143  |
| Protein SET                                  | SET        | 3.14                | .011534  |
| Protein CutA                                  | CUTA       | 3.09                | .0021038 |
| Peptidyl-prolyl cis-trans isomerase A         | PPIA       | 3.09                | .0071350 |
| Galectin-1                                   | LGALS1     | 3.08                | .0000155 |
| Protein disulfide-isomerase                   | P4HB       | 3.08                | .0009140 |
| F-actin-capping protein subunit α-1           | CAPZA1     | 3.05                | .0000032 |
| Histone H2B type 1-L                         | HIST1H2BL  | 3.01                | .0104479 |
| Thrombospondin-3                             | THBS3      | 3.01                | .0061737 |
| α-actinin-4                                  | ACTN4      | 2.99                | .0006276 |
| Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 | PLOD1     | 2.99                | .000104  |
significantly triggers DNA damage (phospho-γ-H2AX index, H2A histone family member X), and did not affect tumor cell proliferation (Ki-67 index) (Figure 6A–C). When exploring drug effects on the stroma, we observed that the combination therapy did not affect the presence of intratumor CD8-positive lymphocytes, or myeloid lymphocyte antigen 6 complex locus G–positive and myeloperoxidase-positive granulocytes, as compared with gemcitabine (Figure 6D–F), but significantly decreased CD206-positive myeloid cell number, a read-out of TAMs (Figure 6G and H). αSMA-positive CAFs and ECM (glycosaminoglycan and collagen) deposits (quantified in turquoise blue/green using the Movat Pentachrome stain) were robustly decreased with the combination therapy, as compared with gemcitabine (Figure 6I–L). Tumor cell STAT3 (signal transducer and activator of transcription member 3) phosphorylation, a reported marker of ECM-induced mechanical stress and of increased tumor cell invasiveness,25 followed the same pattern as ECM deposits (Figure 6M). Gemcitabine tends to increase vessel area (using a monoclonal mouse-specific panendothelial cell antigen antibody [MECA32]), and to decrease pericyte recruitment around tumor vessels (as quantified using the co-stain of MECA32 and neuron-glial antigen 2 [NG2]), effects that are significantly reversed when SOM230 is combined (Figure 6N–P). Interestingly, pericyte depletion was reported to induce metastasis.26 In liver metastases, CD206-positive myeloid cell number also was decreased by the combination therapy as compared with gemcitabine (Figure 7A and B), as well as αSMA-positive cells (Figure 7C and D), whose number increased with metastasis size (Figure 7E). Altogether, our results show in KPC mice that SOM230 normalizes the immune monocytic, fibrotic, and vascular stroma, in correlation with its anti-metastatic potential.

When searching for factors commonly down-regulated by SOM230 in the CAF secretome (Table 1), in the plasma of drug-treated grafted mice (Table 2), and in the plasma of KPC mice also treated with gemcitabine (Figure 7F–H, Table 1. Continued

| Protein names | Gene names | Moy ratio NT/SOM230 | P value |
|---------------|------------|---------------------|---------|
| Acylamino-acid–releasing enzyme | APEH | 2.97 | .0382093 |
| Lysyl oxidase homolog 2 | LOXL2 | 2.96 | .0002226 |
| Thrombospondin–4 | THBS4 | 2.92 | .017107 |
| Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 | PLOD2 | 2.90 | .0165680 |
| Glyceraldehyde-3-phosphate dehydrogenase | GAPDH | 2.89 | .000848 |
| Calmodulin | CALM1 | 2.89 | .0223212 |
| X-ray repair cross-complementing protein 5 | XRCC5 | 2.88 | .0123259 |
| Phosphoglycerate kinase 1 | PGK1 | 2.81 | .0111379 |
| 14-3-3 protein γ | YWHAG | 2.77 | .0000012 |
| Collagen α-1(IV) chain | COL6A1 | 2.72 | .001905 |
| Rho guanine nucleotide exchange factor 18 | ARHGEF18 | 2.66 | .0000016 |
| Malate dehydrogenase, cytoplasmic | MDH1 | 2.66 | .0185247 |
| Triosephosphate isomerase | TPI1 | 2.63 | .0036592 |
| Fructose-bisphosphate aldolase A | ALDOA | 2.59 | .0288449 |
| Vinculin | VCL | 2.54 | .0021035 |
| α-enolase | ENO1 | 2.51 | .0004602 |
| Apolipoprotein C-III | APOC3 | 2.46 | .0180784 |
| Secernin-1 | SCRN1 | 2.45 | .0288882 |
| Ubiquitin-60S ribosomal protein L40 | UBA52 | 2.39 | .0001226 |
| 14-3-3 protein β/α | YWHAB | 2.38 | .0002045 |
| DnaJ homolog subfamily C member 13 | DNAJC13 | 2.38 | .0120707 |
| 14-3-3 protein epsilon | YWHAE | 2.37 | .0002483 |
| Moesin | MSN | 2.35 | .0003671 |
| Phosphoglycerate mutase 1 | PGAM1 | 2.24 | .0024083 |
| Nicotinamide N-methyltransferase | NNMT | 2.22 | .0153174 |
| Ferritin heavy chain | FTH1 | 2.20 | .0224213 |
| Aldose 1-epimerase | GALM | 2.18 | .0006598 |
| Nuclear receptor subfamily 1 group D member 2 | NR1D2 | 2.02 | .0248565 |

NOTE. n = 9 CAF primary cultures, P < .05. IQGAP1, IQ Motif Containing GTPase Activating Protein 1; NSF, N-Ethylmaleimide-Sensitive Factor.
Table 4), we again retrieved CSF-1 among 5 other proteins, also including periostin (Figure 7H). Hence, in KPC mice, SOM230 reduced plasma or intratumor CSF-1 expression when combined with gemcitabine compared with gemcitabine alone (Figure 7I and J). Interestingly, CSF-1 plasmatic concentration in KPC mice correlated with the extent of liver metastatic load (Figure 7K). In KPC primary tumors, αSMA-positive CAFs, but barely tumor cells, expressed CSF-1 (Figure 7L). However, in KPC liver metastases, CSF-1 was expressed heterogeneously, mainly in stromal cells (cytokeratin-19-negative but also αSMA-negative) in large metastases (>0.7 mm²) (Figure 7M, 3 upper panels), but mainly in tumor cells (cytokeratin-19-positive), with a membranous localization, in smaller metastases (<0.7 mm²)
Cancer cells-PSC grafted mice

Plasmatic proteins Cancer-cells-PSC grafted mice

D) CAF

E) CSF-1

Human PDAC (1)

Human PDAC (2)

F) Plasmatic CSF-1

G) CSF-1

Akt

NT SOM230

H) CSF-1

I) Tumor volume

J) Lung metastasis incidence (%)

K) H&E

NT SOM230

Cancer cells PSC WT PSC CSF-1 SOM230
(Figure 7M, 3 lower panels). Altogether, in a spontaneous PDA murine model, these results confirmed that SOM230 provides a therapeutic antimitastatic benefit when given in combination with gemcitabine, in correlation with a drug inhibitory effect on the chemokine CSF-1. Our results also show that the CSF-1 plasmatic concentration correlates with the metastatic potential in KPC mice, and that CSF-1 is expressed mainly in the stroma in KPC primary tumors and large stroma-rich metastases.

**CSF-1 Is a Stromal Marker of Patient PDA Aggressiveness and Metastasis**

To link CSF-1 expression with clinical parameters and biological tumor phenotypes in PDA patients, and because CSF-1 mRNA may be expressed by both human tumor cells and stromal CAFs, we investigated 2 public RNA sequencing (RNaseq) databases, in which epithelial and stromal genes are quantified separately: first, the Maurer et al database, in which RNaseq were run on patient PDA microdissected-stromal and epithelial lesions (n = 65 patients) (Figure 8A–G); and, second, the patient-derived xenograft RNAseq (PaCaOmics patient-derived xenografts [PDX], n = 30), originating from the human and mouse gene sequences, originating from the tumor’s epithelial or stromal compartment, respectively, are distinguishable (Figure 8H–L). In both databases, we found CSF-1 mRNA to be significantly more abundant in the stroma than in the epithelial compartment (Figure 8A and H). Moreover, high levels of stromal CSF-1 mRNA correlated significantly with high American Joint Committee on Cancer tumor staging (patient cohort) (Table 5), or with clinical parameters of disease progression, that is, nonresectability status (PDX cohort) (Figure 8J), which is not the case for epithelial CSF-1 mRNA (Table 5, Figure 8J). Moleculaly, high stromal CSF-1 mRNA expression correlated positively with signatures of aggressive “stroma-activated” (Figure 8B) and “epithelial basal-like” tumors (Figure 8C and K), and negatively with more-differentiated “epithelial classical-like” signatures (Figure 8D). High epithelial CSF-1 mRNA expression also was associated significantly with stroma-activated signatures (Figure 8E), but not consistently with signatures of the tumoral epithelium (basal, classic) because the link was observed in PDX (Figure 8L), but not in microdissected, lesions (Figure 8F and G). Such correlative analyses then were validated using a third patient cohort (PDA resected tissues from n = 38 patients) in which a CSF-1 protein immunohistochemistry score was quantified in both the tumor epithelial and stromal compartments (Figure 8M–T). CSF-1 protein was expressed in both the epithelial and stromal compartments, the latter also was aSMA positive (Figure 8M). Clinically, patients with a high metastatic lymph node ratio (LNR) presented with significantly higher stromal, but not epithelial, CSF-1 scores (Figure 8N). Accordingly, patients with high stromal CSF-1 scores tended to have a shorter life expectancy (P = .11) (Figure 8X), which was not the case for high epithelial CSF-1 patients (Figure 8Y). Pathologically, tumors classified with a high activated stroma index or CD163 (TAM marker) scores also presented with significantly increased stromal, but not epithelial, CSF-1 scores (Figure 8O and P). Conversely, CD8 scores were not linked to either stromal or epithelial CSF-1 scores (Figure 8Q). Molecularly, high stromal or epithelial CSF-1 scores correlated positively with stromal RNaseq signatures of activated stroma or of activated/inflamed stroma, respectively (Figure 8R and S), whereas high stromal CSF-1 scores only correlated negatively with RNaseq signatures of inactivated/structured stroma (Figure 8T) and of epithelial classic-like tumors (Figure 8V). No correlation was found between stromal or epithelial CSF-1 scores with RNaseq signatures of immune stroma (Figure 8U) or of epithelial basal-like tumor (Figure 8W). Altogether, these results show that a high stromal CSF-1 score correlated consistently with stromal activities recognized as being of

**Figure 3.** (See previous page). SOM230 reduces tumoral and plasmatic CSF-1 expression dependently on CAF-expressed sst1, explaining its anti metastatic effect in murine PDA models. (A) Membrane antibody arrays using plasma from mice grafted with cancer cells (CCs) plus PSCs, and treated or not (NT) with SOM230 (pool of n = 5 mice/membrane). (B) Cytokine array quantification of plasma proteins in CCs plus PSC grafted mice and treated with SOM230 as compared with untreated (NT), showing down-regulated proteins (pool of n = 5 mice/group). (C) Venn diagram of the overlap of down-regulated plasma proteins from grafts identified in cytokine arrays (A, B) and from CAF secreteome analysis (Table 1). Common proteins are listed underneath. (D and H) CSF-1 ELISA quantification in (D) conditioned media of human CAF (n = 5 CAF primary cultures), or (H) murine PSC overexpressing CSF-1 or not (mock) (performed in quadruplicate), treated or not (NT) with SOM230, and expressed relative to NT. The paired or unpaired Student t test was used. (E) Immunofluorescence confocal analysis of CSF-1 (green), sst1 (red), and cytokeratin-19 (CK-19; purple) expression in 2 patient-derived PDA tissues, showing co-localization in the stroma (at distance from tumor epithelial CK-19-positive cells) of sst1 and CSF-1. Scale bars: 50 μm. (F) Plasma CSF-1 ELISA quantification at death of ungrafted mice (first condition), or grafted mice treated or not with SOM230 (Figure 2, n = 4 per group). Each dot represents the CSF-1 plasma concentration measured in 1 mouse. Analysis of variance followed by the Bonferroni multiple comparison post-test was used to generate P values. (G) Western blot analysis of tumor lysates from grafted mice (from Figure 2, mouse tumors 1-to-6) treated or not (NT) with SOM230 (n = 6 mice/treatment condition), with anti–CSF-1 or Akt (loading control) antibody, and quantification (densitometric analysis) of CSF-1 normalized to Akt expression (A.U., arbitrary unit). The unpaired Student t test was used to generate P values. (I–K) CCs were syngeneically and orthotopically co-grafted with murine PSCs overexpressing or not (MOCK) a secreted CSF-1 form (PSC-CSF-1). After 3 weeks, mice were randomized and treated or not (NT) with SOM230-LAR for 3 weeks. After 6 weeks, mice were killed (n = 6 mice/group). (J) Tumor volume evolution from treatment day 1 to 21, monitored weekly by ultrasound. (J) Metastasis incidence in mouse lungs and distribution of mice according to their metastatic lung load (cumulative metastatic lung area less than or greater than 0.1% of the total lung area). The chi-squared test was used to generate P values. (K) Representative H&E staining of mouse lungs. Dashed lines encircle metastases. Scale bars: 100 μm. *P < .05, **P < .01, and ***P < .001
poor prognosis for PDA patients,\textsuperscript{4,6} alongside increased tumor cell aggressiveness (less-differentiated phenotype), which, however, was not the case for the epithelial CSF-1 score.

### Table 2. Listing of Down-Regulated Proteins in Cancer Cell–PSC Grafted Mice Plasma: SOM230 vs Untreated

| Protein name          | Log ratio SOM230/NT |
|-----------------------|---------------------|
| CD14                  | -0.836393           |
| IFN-γ                 | -0.257125           |
| TIM-1                 | -0.2522933          |
| IL5                   | -0.2357543          |
| Chemerin              | -0.2076414          |
| PDGF-BB               | -0.2058304          |
| IL10                  | -0.2005681          |
| Leptin                | -0.1993592          |
| Serpin F1             | -0.1981243          |
| F7-3 ligand           | -0.1887131          |
| CD160                 | -0.1865666          |
| CXCL9                 | -0.1772411          |
| TNF-α                 | -0.1492109          |
| Myeloperoxidase       | -0.1471288          |
| Pentraxin 2           | -0.1347746          |
| E-selectin            | -0.1293948          |
| IL12p40               | -0.121981           |
| FGF acidic            | -0.1169429          |
| IL13                  | -0.1077902          |
| LDL R                 | -0.1072964          |
| VCAM-1                | -0.1065818          |
| IL11                  | -0.09950715         |
| PD-ECGF               | -0.0970886          |
| M-CSF                 | -0.09599823         |
| RBP4                  | -0.095954           |
| LIF                   | -0.09346522         |
| PCSK9                 | -0.09068561         |
| CCL11                 | -0.08666945         |
| IL1α                  | -0.08475557         |
| IGFBP-3               | -0.08151417         |
| IL27p28               | -0.0749929          |
| CXCL11                | -0.07498571         |
| RAGE                  | -0.06808221         |
| CXCL10                | -0.05732954         |
| Pentraxin 3           | -0.05685075         |
| Endoglin              | -0.05549461         |
| IL7                   | -0.05403424         |
| Pref-1/DLK-1          | -0.0525299          |
| P-selectin            | -0.05165642         |
| IGFBP-5               | -0.04817382         |
| IL15                  | -0.04627419         |
| Periostin             | -0.04173948         |
| Lipocalin-2           | -0.03777582         |

### Discussion

CAFs are master players in the secretion of proteins regulating the tumor microenvironment. Through secreto-meme MS analyses on media conditioned by 9 primary CAF cultures, here we identified CAF-secreted proteins, which are under the negative control of SOM230. Among them, 47 were found commonly in stroma-activated signatures published for human PDA.\textsuperscript{4,6} Such concurrent inhibition by SOM230 of several CAF protumoral signals was consistent with the robust antimetastatic effect that we obtained in 2 different immunocompetent mouse PDA models, and most certainly provided a therapeutic advantage in comparison with specific pharmacologic inhibitors or blocking antibodies, which are directed against 1 specific factor or receptor. This is particularly essential in PDA, in which many single-target drug therapies thus far have failed, even when combined with chemotherapies (eg, hedgehog pathway inhibitor,\textsuperscript{29} or pegvorhyaluronidase alfa [PEGPH20]).\textsuperscript{30} A combination of several drugs seems necessary to reach efficacy in PDA, at the cost of safety and adverse effects for patients. SOM230 represents a safe drug (Food and Drug Administration–approved for neuroendocrine Cushing tumors and safely tested in a phase-I PDA trial in combination with gemcitabine\textsuperscript{31}), with a pharmacologic inhibitory action on stt1-expressing CAFs, abrogating CAF cross-talk with tumor and other stromal cells.

Why SOM230 alone is not as effective at inhibiting metastasis in KPC mice as when it is combined with gemcitabine is intriguing. Gemcitabine was reported to alter the pancreatic immune, fibrotic, and vascular stroma\textsuperscript{19,32,33} de novo triggering a metastatic microenvironment.\textsuperscript{34,35} SOM230 did not improve gemcitabine-induced cytotoxicity in tumor cells, but normalized the gemcitabine-remodeled stroma, reducing stellate cell activity, tumor-associated macrophage presence, ECM deposits, and angiogenesis.
**Figure 4. SOM230 reduces TAM presence.** (A and B) Experimental design of the study. (A) Cancer cells were syngeneically and orthotopically co-grafted with murine WT or KOsst1 PSCs. One week after graft, mice were randomized and treated or not with SOM230-LAR. Three weeks after graft, mice were killed. (B) Gating strategy to identify viable cells, total CD45-positive cells, total macrophages, CD206high macrophages, and myeloid-derived suppressor cell immune populations (i, red boxes), or total CD45-positive cells, total lymphocytes, helper CD4 lymphocytes, and cytotoxic CD8 lymphocytes immune populations (ii, blue boxes). (C–I) Proportions of (C) total immune cells (CD45+ cells), (D) total macrophages (CD45+; F4/80+; CD11b+ cells), (E) CD206hi macrophages, (F) myeloid-derived suppressor cell (CD45+; CD11b; lymphocyte antigen 6 complex locus G [Ly6g]+ cells), (G) T lymphocytes (CD45+; CD3+ cells), (H) helper T lymphocytes (CD4+ T lymphocytes), and (I) cytotoxic T lymphocytes (CD8+ T lymphocytes) were quantified (in % of total cells) by flow cytometry on tumors from cancer cells + PSC-injected mice (n = 4–5 mice/group). The Kruskal–Wallis test followed by the Dunn multiple comparison post-test was used to generate P values. *P < .05.
Table 3. Characteristics of KPC Mice

| Drug ID | Age at treatment start, d | Tumor volume on treatment day 1, mm³ | Tumor volume at death, mm³ | Survival, d | Liver metastasis | Liver metastatic area, % | Ascites |
|---------|---------------------------|-------------------------------------|---------------------------|-------------|------------------|--------------------------|---------|
| No 30   | 203                       | 150                                 | ~1000                     | 21          | Yes              | 0.02                     | Yes     |
| No 72   | 203                       | 146                                 | ~1000                     | 35          | Yes              | 0.24                     | No      |
| No 113  | 161                       | 34                                  | ~1000                     | 42          | No               | 0.00                     | No      |
| No 200  | 133                       | 66                                  | ~1000                     | 35          | Yes              | 0.11                     | No      |
| No 748  | 131                       | 167                                 | ~1000                     | 30          | Yes              | 4.72                     | Yes     |
| No 735  | 175                       | 40                                  | ~1000                     | 21          | Yes              | 6.94                     | Yes     |
| No 244  | 210                       | 113                                 | ~1000                     | 20          | Yes              | 36.40                    | Yes     |
| No 1002 | 114                       | 115                                 | ~1000                     | 23          | Yes              | 4.98                     | Yes     |
| SOM230 828 | 205                 | 145                                 | ~1000                     | 21          | Yes              | 0.06                     | No      |
| Gemcitabine 862 | 210                 | 101                                 | ~1000                     | 16          | No               | 0.00                     | No      |
| Gemcitabine 1027 | 164                | 53                                  | ~1000                     | 56          | No               | 0.00                     | No      |
| SOM230 824 | 247                 | 41                                  | ~1000                     | 52          | Yes              | 2.92                     | No      |
| SOM230 954 | 238                | 170                                 | ~1000                     | 22          | Yes              | 5.79                     | Yes     |
| SOM230 719 | 143                 | 113                                 | ~1000                     | 21          | Yes              | 10.74                    | Yes     |
| SOM230 734 | 156                 | 99                                  | ~1000                     | 21          | No               | 0.00                     | No      |
| SOM230 690 | 126                 | 72                                  | ~1000                     | 24          | Yes              | 7.35                     | No      |
| Gemcitabine 310 | 162                 | 200                                 | ~1000                     | 20          | Yes              | 28.15                    | No      |
| Gemcitabine 222 | 162                 | 31                                  | ~1000                     | 37          | Yes              | 0.04                     | No      |
| Gemcitabine 269 | 172                 | 149                                 | 560                       | 50          | Yes              | 59.03                    | No      |
| Gemcitabine 462 | 205                 | 195                                 | ~1000                     | 46          | Yes              | 11.89                    | Yes     |
| Gemcitabine 488 | 273                 | 108                                 | ~1000                     | 13          | Yes              | 9.75                     | Yes     |
| Gemcitabine 947 | 204                 | 144                                 | ~1000                     | 21          | No               | 0                        | Yes     |
| Gemcitabine 901 | 233                 | 50                                  | 700                       | 60          | Yes              | 1.44                     | No      |
| Gemcitabine 1096 | 148                | 53                                  | ~1000                     | 80          | Yes              | 5.99                     | No      |
| Gemcitabine SOM230 104 | 210                 | 158                                 | ~1000                     | 31          | No               | 0.00                     | No      |
| Gemcitabine SOM230 254 | 145                 | 123                                 | ~1000                     | 39          | Yes              | 0.05                     | No      |
| Gemcitabine SOM230 125 | 267                 | 190                                 | ~1000                     | 32          | Yes              | 0.46                     | No      |
| Gemcitabine SOM230 140 | 192                 | 32                                  | 800                       | 91          | No               | 0.00                     | No      |
| Gemcitabine SOM230 194 | 212                 | 80                                  | ~1000                     | 42          | Yes              | 2.86                     | Yes     |
| Gemcitabine SOM230 953 | 194                 | 90                                  | ~1000                     | 77          | No               | 0.00                     | No      |
| Gemcitabine SOM230 660 | 197                 | 111                                 | ~1000                     | 37          | Yes              | 0.35                     | Yes     |
| Gemcitabine SOM230 872 | 150                 | 78                                  | ~1000                     | 51          | No               | 0.00                     | Yes     |

We focused on the CSF-1 chemokine, which was down-regulated by SOM230 in CAF secretomes and in PDA harboring mouse plasma. CSF-1 is involved in intratumor recruitment of monocytes, and in their polarization into macrophages, endowed with pro-angiogenic and ECM remodeling features that favor tumor cell metastasis. In PDA patients, high serum CSF-1 levels were correlated with advanced tumor stages. Accordingly, we observed that the plasma CSF-1 level correlated with liver metastatic load in KPC mice. SOM230 treatment decreased intratumor and plasma CSF-1 levels, consistent with fewer intratumor macrophages. In vitro, CAFs were reported to cross-talk with macrophages. In vivo, CAFs were shown to secrete CSF-1, which was increased in the plasma of KPC mice. In summary, SOM230 delays the growth and metastasis of spontaneous pancreatic tumors generated in the immunocompetent KPC mouse model. (A) Representative immunostaining of αSMA and sst1 receptor in serial sections of a KPC primary tumor (untreated mice) showing expression of sst1 receptor in αSMA-positive cell areas. Scale bars: 200 μm. (B–H) Experimental design. (B) KPC mice had an ultrasound weekly from 8 weeks of age until tumor size enrollment was reached (homogeneous treatment group tumor volumes, 31–200 mm³; mean, 103.9 mm³; analysis of variance followed by the Bonferroni multiple comparison post-test was used). (C) Mice were randomized into treatment groups (PBS-treated [NT], SOM230-long-acting release [LAR], gemcitabine [Gem], or gemcitabine plus SOM230-LAR [Gem-SOM230] n = 8 mice per group). (D–H) Tumor volume was quantified by ultrasound until ethical end point (when maximal-authorized volume of the primary tumor, or clinical end points, were reached). Tumor volume progression from treatment day 1 to 21. Analysis of variance followed by the Bonferroni multiple comparison post-test was used to generate P values. (D) Representation of tumor volume progression from treatment day 1 until death for each mouse in the 4 treatment conditions. (E–H) KPC mouse identity numbers are indicated, according to Table 3. (I) Tumor weight was measured at euthanasia (n = 4–5 mice/group) (analysis of variance followed by the Bonferroni multiple comparison post-test was used to generate P values). (J) Survival analysis (Kaplan–Meier) was analyzed using a log-rank test. (K–M) KPC liver metastasis and mouse survival analyses. (K) Representative H&E staining. Arrows indicate metastases. Scale bars: 2.5 mm. Metastasis incidence in KPC livers and distribution of mice according to their metastatic liver load (cumulative metastatic liver area less than or greater than 3% of the total liver area) was determined with the chi-squared test. (M) Survival analysis of KPC mice treated as indicated. Kaplan–Meier survival data were analyzed using a log-rank test. *P < .05, **P < .01, and ***P < .001.
**A** Tum. NT SOM230 Gem Gem-SOM230

**B**

**C** Tum. NT SOM230 Gem Gem-SOM230

**D** Tum. NT SOM230 Gem Gem-SOM230

**E** Tum. NT SOM230 Gem Gem-SOM230

**F** Tum. NT SOM230 Gem Gem-SOM230

**G** Tum. NT SOM230 Gem Gem-SOM230

**H**

**I**

**J**

**K**

**L**

**M**

**N**

**O** Tum. NT SOM230 Gem Gem-SOM230

**P**

**Q**
with macrophages pushing toward the M2 phenotype through CAF-derived CSF-1.\textsuperscript{12,38} We showed that the SOM230 antimitostatic effect relied on CAF-expressed st1t1, and on the drug inhibitory effect of CAF-secreted CSF-1.

In preclinical studies, CSF-1 receptor (CSF-1-R) blockade using CSF-1-R inhibitors or blocking antibodies resulted in a shift from mostly immunosuppressive to improved antigen presentation capacities of tumor-associated macrophage subsets, with a therapeutic benefit for different solid tumors including PDA, when associated with chemotherapy or radiotherapy.\textsuperscript{6} In PDA, the CSF-1-R blockade decreased tumor growth through increased intratumor cytotoxic CD8 T-cell recruitment,\textsuperscript{27} and synergized with checkpoint-based immunotherapies.\textsuperscript{40} SOM230 alone or in combination with gemcitabine did not increase intratumor CD8 T-cell recruitment in 2 PDA mouse models, even though it decreased plasma and intratumor CSF-1, and was antimitostatic. CSF-1-R blockade recently was shown to trigger transcriptomic reprogramming of PDA tumor cells to a more differentiated and less-aggressive PDA subtype,\textsuperscript{11} stressing the critical role of CSF-1-R–expressing macrophages in driving PDA tumor cell plasticity, in addition to their role in blocking T-cell responses. This is consistent with our observation that CSF-1 mRNA or protein expression, when quantified specifically in the stroma, correlates with markers of aggressiveness, that is, clinical markers (tumor stage, LNR), pathologic markers (activated stroma index, CD163-positive macrophage presence), and molecular markers (activated status of the stroma, increased basal-like and/or decreased classic-like tumor cell phenotype). However, we could not correlate epithelial CSF-1 mRNA or protein expression with an inactivated status of the stroma as it was recently reported in pure (stroma-poor) basal-like tumors.\textsuperscript{1} A possible explanation is that our immunohistochemistry PDA cohort did not include rare pure basal-like tumors. Indeed, it comprised 29% of basal-like tumors and 71% of classic-like tumors, which harbor either an activated stroma (19% and 17%, respectively) or a desmoplastic stroma (10% and 32%, respectively), or poor stroma content for 22% of pure classic tumors, as previously described.\textsuperscript{6} We observed that epithelial CSF-1 expression correlated with the presence of CD163-positive macrophages and with a molecular “activated/inflamed” stroma status recognized to be of bad prognosis in classic tumors.\textsuperscript{3} PDA stroma seemed to be the principal source of CSF-1, at least when quantified at the mRNA level in 2 independent PDA cohorts, which was consistent with a recently described CSF-1–expressing immunomodulatory CAF subset identified in melanomas.\textsuperscript{66} CSF-1 protein quantification (by immunohistochemistry) from a third PDA cohort indicated a higher epithelial rather than stromal source, although this may have been biased by differences in antibody recognition of the membranous (uncleaved) and soluble (cleaved) CSF-1 forms, which could be distributed differently in the epithelial and stromal compartments (eg, we observed a membranous expression of CSF-1 in KPC liver metastatic tumor cells) (Figure 7M). How and where the membranous CSF-1 protein is cleaved, releasing the soluble chemotactant factor, and whether membranous and soluble CSF-1 have similar functions in tumors, are as yet unresolved but important issues to understand.

Altogether, our results show that antimitostatic SOM230 pharmacotherapy should benefit PDA patients enrolled in chemotherapeutic protocols, the effect of SOM230 could be explained mechanistically by a broad inhibitory action on CAF cross-talk with tumor and nontumor cells, including macrophages through CSF-1.

**Methods**

**Cell Lines and Primary CAF Cultures**

KPC cells were derived from PDA tumor tissue obtained from LSL-KrasG12D/+; LSL-Trp53R172H/+; p48-Cre−/− mice (C57BL/6 background, a generous gift from Professor D. Saur, Technische University of Munich). PSCs were isolated from mouse pancreata, either WT or KOsSt1 (a generous gift from Dr C. Viollet, INSERM U1266, Institute of Psychiatry and Neuroscience of Paris)\textsuperscript{20} (C57BL/6 background), as described.\textsuperscript{43} In brief, pancreata were minced and digested for 30 minutes at 37°C in a dissociation buffer containing 0.05% collagenase P (Sigma-Aldrich, Lyon, France), 0.1% DNase I (Sigma-Aldrich), and 0.02% Pronase (Sigma-Aldrich) in Grey’s balanced salt solution (GBSS; Sigma-Aldrich). Digested pancreata were filtered through a 100-μm nylon mesh and washed in GBSS with 0.3% bovine serum albumin (BSA). After spinning, the cell pellet was resuspended in 12 mL GBSS with 0.3% BSA and 10 mL 43.75% Nicodenz (Sigma-Aldrich). A total of 11 mL cell suspension was layered below 4.5 mL of GBSS with 0.3% BSA, and the gradient was centrifuged for 20 minutes at 2600 rpm (with break switched off). The cells in the fuzzy band just above the interface between the Nicodenz and

![Figure 6](See previous page). Macrophage polarization, ECM deposit, and angiogenesis are decreased in primary KPC tumors upon combination treatment as compared with gemcitabine. Quantification and representative pictures of markers analyzed by immunohistochemistry using the indicated antibodies in KPC primary tumors (Tum.). Each value represents the marker quantification in each tumor (n = 8 mice/group). (A and B) Phospho–γH2AX–positive cells (in % of total cells). Scale bars: 50 μm. (C–F) Quantification of (C) Ki-67–positive cells (in % of total cells), and of (D) CD8-positive cells, (E) lymphocyte antigen 6 complex locus G [Ly6G]–positive cells, or (F) myeloperoxidase (MPO)–positive cells (number/field). (G and H) CD206 mean fluorescence intensity (in intensity of each field). Scale bars: 100 μm. (I and J) αSMA–positive area (in % of each total field area). (K and L) Turquoise blue/green-positive area from Movat pentachrome staining (in % of each total field area). (M) Quantification of phospho–STAT3–positive cells (in number/mm²). (N) Representative pictures. (O) Quantification of MECAS32–positive cells (in % of 4,6-diamidino-2-phenylindole [DAPI]–positive cells) (n = 4–6 mice/group). (P) Quantification of NG2/MECA32 double-positive cells (% of NG2–positive pericytic cells in the Meca32–positive cell population, as in \textsuperscript{46} (n = 4–6 mice/group). Kruskal–Wallis test followed by the Dunn multiple comparison post-test was used to generate \(P \) values. *\( P < .05 \), **\( P < .01 \). p–STAT3, phospho-signal transducer and activator of transcription.
GBSS were harvested, washed in phosphate-buffered saline (PBS), and plated. PSCs were transduced with a lentivector expressing a full-length human CSF-1 (a gift from M. Roussel and C. Sherr, plasmid 86797; Addgene, Watertown, MA). Lentivector pLoc CSF1-181 Ires TGFP Blast was obtained by ligating digested polymerase chain reaction (PCR) product of CSF-1 (AA 1-181) with into BamHI/Nhel-digested pLoc-Ires tGFP Blast (Dharmacon, Horizon Discovery LTD, Cambridge UK). CAFs were isolated from human pancreatic tumor tissues by outgrowth, using the explant techniques from histologically fibrotic areas of pancreas surgically resected from PDA patients. Human pancreatic tumor tissues were obtained from the Pathology Department of Limoges Hospital (Limoges, France), from patients undergoing pancreatic resections for pancreatic adenocarcinoma (convention CRB/MAD-CC-2013-002). Small tissue blocks were cut (0.5–1 mm²) using a razor blade and were seeded in 10 cm² uncoated culture wells in the presence of Dulbecco’s modified Eagle medium–F12 containing 10% fetal calf def-complemented serum. Tissue blocks were cultured at 37°C in a 5% CO₂-air humidified atmosphere. Eighteen hours after seeding, culture medium was changed. CAFs grew out from the tissue blocks 1–3 days later. An authorization for collecting and conserving this collection was given by the Comité de Protection des Personnes Sud-Ouest et Outre-Mer II (Déclaration de Conservation et Préparation de Collections DC-2016-2654). This study was approved by the Ethic Committee of the Institution.

Secretome Liquid Chromatography MS/MS Analysis

Ten to 15 mL of secretomes from CAFs (from n = 9 tumor patients), treated or not for 48 hours with SOM230 (10⁻⁷ mol/L), and from CAF culture immortalized with hTERT (human telomerase reverse transcriptase [CAFhTERT]), were analyzed in biological duplicates by nano-liquid chromatography-MS/MS using an Ultimate3000 system (Dionex, Thermofisher Scientific) coupled to an Orbitrap Tribrid Fusion mass spectrometer (Thermo Fisher Scientific). To take into account the variabilities arising from secretome biochemical preparation and liquid chromatography MS/MS analyses, normalization across the compared samples was performed by adjusting the medians of the distribution of heavy-labeled protein intensities arising from CAFhTERT (used as an internal standard) for all runs. To do so, a primary CAFhTERT culture was labeled with heavy amino acids (L-arginine and L-lysine) through stable labeling with amino acids in cell culture. Proteins with a fold change of more than 2 and a Student t test P value less than .05 were reported as potentially regulated secreted proteins under SOM230 treatment.

Animal Models

Transgenic mice. KPC, LSL-KrasG12D/+; Ink4aR16/+; Pdx-1-Cre1/−, and KOsst1 mice have been described previously.

KPC model. Because the kinetic of tumor onset is heterogeneous in the KPC model, mice were enrolled when 30–200 mm³ tumor was detected by weekly ultrasonography monitoring (Vevo 2100; Fujifilm VisualSonics Amsterdam NL, or Aixplorer; Supersonic Imagine Aix-en-Provence France), as indicated (Figure 5B). Treatments were started at homogeneous tumor sizes (Figure 5C). Mice were treated subcutaneously with SOM230-LAR (80 mg/kg, once every 28 days) and/or intraperitoneally with gemicitabine (100 mg/kg, twice per week). Mouse ages and tumor volumes at treatment start and death (ethical end points) are reported (Table 3, Figure 5B and C). All mice were killed when primary tumor volume reached 1 cm³, or when mice developed ethical clinical end points (Table 3). Age at death was not statistically different between treatment groups (Table 3), except for untreated mice, which lived for a shorter length of time. Survival events were scored when 1 ethical end
point was reached (tumor burden >1000 mm³, or body weight loss >20%, or reaching any clinical end point defined by our institutional guidelines and European animal protection law).

**Orthotopic graft models.** Age-matched 8-week-old female C57BL/6J mice were used (Charles River Laboratories, Ecully France). Syngeneic orthotopic tumors were established by surgical implantation of a 1:3 mix of pancreatic cancer KPC cells (2.10⁴) and PSCs (6.10⁴). Mice were treated

### Table 4. Listing of Down-Regulated Proteins in KPC Plasma: Gemcitabine-SOM230 Vs Gemcitabine

| Protein names   | Log ratio Gem-SOM230/Gem |
|-----------------|--------------------------|
| Osteoprotegerin | -0.4554106               |
| WISP-1          | -0.3132871               |
| CD14            | -0.2991138               |
| Myeloperoxidase | -0.2927159               |
| Serpin F1       | -0.2775624               |
| Angiopoietin-2  | -0.2744032               |
| CCL21           | -0.2670901               |
| Endoglin        | -0.2538754               |
| Serpin E1       | -0.2071946               |
| MMP-3           | -0.1905704               |
| M-CSF           | -0.1955245               |
| CCL22           | -0.1905704               |
| BAFF            | -0.1831733               |
| Adiponectin     | -0.1755342               |
| CCL17           | -0.1687388               |
| Gas 6           | -0.1684436               |
| IL23            | -0.1624154               |
| TNF-α           | -0.1563117               |
| Proliferin      | -0.1550597               |
| LDL R           | -0.1511058               |
| IL17A           | -0.1482403               |
| C1q R1          | -0.1431448               |
| Pref-1          | -0.1390524               |
| E-selectin      | -0.1310115               |
| IGFBP-3         | -0.1281087               |
| VEGF            | -0.1134834               |
| CD160           | -0.1099336               |
| IL6             | -0.1047299               |
| MMP-9           | -0.1046919               |
| CXCL13          | -0.1005212               |
| IL3             | -0.0973246               |
| MMP-2           | -0.0957026               |
| Periostin       | -0.0931533               |
| PCSK9           | -0.09101482              |
| Osteopontin     | -0.08783475              |
| IGFBP-5         | -0.08713286              |
| CXCL16          | -0.08664412              |
| Complement C5   | -0.08671959              |
| CCL6            | -0.08508948              |
| CD40            | -0.08502961              |
| CXCL1           | -0.08414122              |
| CX3CL1          | -0.08342186              |
| Pentraxin 3     | -0.08190633              |
| IGFBP-2         | -0.07113896              |
| RBP4            | -0.06947069              |
| IL15            | -0.0686098               |
| Thrombopoietin  | -0.06512675              |
| TIM-1           | -0.06420811              |
| GDF-15          | -0.06296449              |

### Table 4. Continued

| Protein names   | Log ratio Gem-SOM230/Gem |
|-----------------|--------------------------|
| VCAM-1          | -0.05980025              |
| IL1β            | -0.05504656              |
| CXCL10          | -0.05379479              |
| ICAM-1          | -0.0524515              |
| Complement factor D | -0.05201          |
| Fetuin A        | -0.05186915              |
| FGF-21          | -0.05006535              |
| Chitinase 3-like 1 | -0.0484128             |
| IL1α            | -0.04661465              |
| EGF             | -0.04635749              |
| IGFBP-6         | -0.04144023              |
| Angiopoietin-like 3 | -0.04035782             |
| PD-ECGF         | -0.03923278              |
| P-selectin      | -0.03810753              |
| Fit-3 ligand    | -0.03448458              |
| IL7             | -0.03444469              |
| Chemerin        | -0.02659499              |
| CCL12           | -0.02624445              |
| CXCL9           | -0.02562579              |
| Endostatin      | -0.02464836              |
| HGF             | -0.01868339              |
| IFN-γ           | -0.0183519               |
| IL1ra           | -0.0171957               |
| DPPIV           | -0.01625448              |
| Lipocalin-2     | -0.01578041              |
| IGFBP-1         | -0.01393292              |
| CXCL11          | -0.01046351              |
| CCL11           | -0.004502624             |

**NOTE.** Pool of n = 5 mice/group.

**BAFF,** B-cell activating factor; **CCL,** CC chemokine ligand; **CXCL,** (C-X-C) motif ligand; **DPPIV,** dipeptidyl-peptidase IV; **EGF,** epidermal growth factor; **FGF,** fibroblast growth factor; **FII-3,** fms like tyrosine kinase 3; **GDF,** glial-derived factor; **HGF,** hepatocyte growth factor; **ICAM-1,** intercellular adhesion molecule-1; **IFN,** interferon; **IGFBP,** insulin-like growth factor binding-protein; **IL,** interleukin; **LDL,** low-density lipoprotein; **MMP,** matrix metalloproteinase; **PCSK9,** proprotein convertase subtilisin/kexin type-9; **PD-ECGF,** platelet-derived endothelial cell growth factor; **RBP,** retinol binding protein; **TIM-1,** T cell Ig and Mucin 1; **TFN,** tumor necrosis factor; **VCAM-1,** vascular cell adhesion molecule 1; **VEGF,** vascular endothelial growth factor; **WISP,** Wnt-1 inducible signaling pathway.
subcutaneously with SOM230-long-acting release (LAR) (80 mg/kg, once every 28 days) and/or intraperitoneally with gemcitabine (100 mg/kg, twice per week) or PBS. SOM230-LAR was provided by Novartis. Gemcitabine was obtained from the Institut Universitaire du Cancer de Toulouse pharmacy and diluted in PBS. Treatments started 3 weeks after grafting when mice were randomly placed in one of the treatment groups (untreated, PBS; and SOM230-LAR–treated). Tumor volumes were measured by ultrasound using the 3-dimensional reconstruction tool (Vevo 2100; VisualSonics), or with the following formula tumor area × tumor diameter × (2/3) (Aixplorer; Supersonic Imagine). For metastasis analysis, formalin-fixed paraffin-embedded lungs or livers were cut (5-μm–thick sections), and 5 slides (every 100 μm) were colored (hematoxylin and eosin) and examined for the presence of metastasis foci. Metastasis foci were identified in each organ section, the corresponding area was quantified, and the metastatic load was calculated as the cumulated area of metastases quantified per section and expressed as the percentage of the total organ area per section, using the NanoZoomer Digital Pathology Virtual Slide Viewer (Hamamatsu Photonics, Massy, France). A cut-off value equal to the calculated median of the cumulative metastatic lung or liver area (percentage of the total organ area) and quantified in the control untreated condition was set to discriminate between mice presenting with a low vs a high metastatic load. All experiments were in accordance with institutional guidelines and European animal protection law and approved by the responsible government agency (agreement number APAFIS21117 201906190061441).

**Colorations and Immunostaining of Mouse Tissues**

Formalin-fixed, paraffin-embedded tumor tissue slides were deparaffinized/rehydrated and stained (H&E or Movat’s pentachrome). For immunostaining, slides subjected to antigen retrieval (10 mmol/L sodium citrate pH 6, or Tris-EDTA pH 8.8 and autoclaved), quenched for peroxidase activity, and blocked using blocking buffer (X0909; Dako, Agilent Technologies, Les Ulis, France). Slides were incubated with the following primary antibodies overnight at 4°C: anti-α-SMA (ab7817, 1/200; Abcam, Amsterdam, NL), anti-CSF-1 (ab99178, 1/200; Abcam), anti-lymphocyte antigen 6 complex locus G (RB6-8C5, ab25377, 1/100; Abcam), antymeloperoxidase (ab188211, 1/500; Abcam), anti-phospho-STAT3 (Tyr705, D3A7, CST9145, 1/200; Cell Signaling Technology, Danvers, MA), anti-Ki-67 (SP6, ab16667, 1/200; Abcam), anti-phospho-γH2AX (Ser139, 20E3, CST9718, 1/500; Signaling Technology), anti-CD8 (D4W2Z, CST98941, 1/400; Signaling Technology), anti-sst1 (a generous gift from Novartis Pharma), now commercialized by Bio-Rad (Marnes la Coquette, France) MCA5924, dilution 1/250). Slides were washed and incubated in ImmunPRESS secondary antibody (mouse MP-7402, rabbit MP7401, or rat MP7404; Vector), and binding was visualized with 3,3’-diaminobenzidine substrate kit (SK4105; Vector, Eurobio Scientific, Les Ulis, France) or aminoethyl carbazole substrate chromogen (K3464; Dako), and counterstained with hematoxylin. Sections were imaged using NanoZoomer XR (Hamamatsu) (service provided by Imag’IN core from the Institut Universitaire du Cancer Toulouse). Quantifications were performed using ImageJ (National Center for Research Resources, National Institutes of Health).

**Figure 8. (See previous page). Stromal CSF-1 is a factor of poor prognosis in human PDA.** (A–G) Analyses of stromal and of tumor epithelial CSF-1 mRNA expression in correlation with functional molecular signatures of PDA stroma and tumor cells, in the Maurer et al27 data set (RNAseq analyses of laser-microdissected PDA lesions (n = 65 tumors) of the tumor epithelium [tumor Epith.] and of the stroma). (A) Differential expression of CSF-1 mRNA in laser-microdissected PDA lesions of the stroma or of the tumor epithelium (tumor Epith.) (n = 65 tumors) from Maurer et al.27 The unpaired t test with Welch correction was used to generate P values. (B and E) GSEA for activated stromal signature, as defined by Moffitt et al,14 or for (C and F) epithelial cancer basal-like signature, or for (D and G) epithelial cancer classic-like signature, in (B–D) high vs low stromal or (E–G) tumor epithelial CSF-1 mRNA-expressing lesions from Maurer et al.27 (H–L) Analyses of stromal and tumor epithelial CSF-1 mRNA expression in correlation with functional molecular signatures of PDA stroma and tumor cells, in the Nicolle et al data set (PaCaOmicS, n = 30 PDX).5 (H) Differential expression of CSF-1 mRNA in stroma (of murine origin) or tumor epithelial (of human origin) cells. The unpaired t test with Welch correction was used to generate P values. (I and J) Correlation between (I) stromal or (J) tumor epithelial level of CSF-1 mRNA with resectability of patients. The Mann–Whitney test was used to generate P values. (K and L) GSEA for pancreatic cancer cell basal signature in high vs low (K) stromal or (L) tumor epithelial CSF-1 mRNA PDX; (M–Y) Analyses of stromal and of tumor epithelial CSF-1 protein expression, in correlation with clinical data of patients, with pathologic data of PDA tumors, and with functional molecular signatures of PDA stroma and tumor cells (RNAseq analyses), in a personal PDA cohort (n = 38 primary tumors). (N) Representative images of CSF-1 and α-SMA expression analyzed by immunohistochemistry on formalin-fixed, paraffin-embedded tissue slides of 2 PDA patient primary tumors (PDA1 and PDA2). (N–W) Quantification using Definiens Tissue Studio of CSF-1 immunohistochemistry (IHC) scores in the stromal (left panels) or in the tumor epithelial (right panels) compartment, and (N) plotted in correlation with a high vs low (based on the median) positive lymph node ratio (LNR), (O) with pathologic markers of the tumors quantified by IHC, that is, activated stroma index (ASI), (P) CD163-positive cells, or (Q) CD8-positive cells. (R–W) RNAseq analyses of the n = 38 PDA tumors generated transcriptomic signature index, as defined by6, of the (R) activated stroma index, (S) activated/inflamed stroma index, (T) inactivated/structured stroma index, and (U) immune stroma index, and of the tumor epithelium, (V) classic-like index or (W) basal-like index, which were compared in CSF-1high vs CSF-1low (based on the median) IHC scores quantified in the stroma (left panel) or in the tumor epithelium (right panel). The unpaired t test was used to generate P values. (X and Y) Survival analysis of patients presenting a (X) high vs low stromal or (Y) tumor epithelial CSF-1 immunohistochemistry score. Kaplan–Meier survival data were analyzed using a log-rank test. *P < .05, **P < .01, and ***P < .001.
Table 5. Links Between Stromal or Tumor Epithelial High Vs Low CSF-1 mRNA Quantified in Microdissected Lesions From the Maurer et al\textsuperscript{27} Patient Cohort (n = 65), With Clinical Features of Patient Disease

|                          | Stroma                              | Tumoral                             |         |
|--------------------------|-------------------------------------|-------------------------------------|---------|
|                          | CSF-1 high (minimum–maximum)        | CSF-1 low (minimum–maximum)         |         |
| Age, mean, y             | 68.4 (48–86)                        | 69.6 (50–85)                        | .614    |
| (minimum–maximum)        |                                     |                                     |         |
| AJCC stage               |                                     |                                     |         |
| 2A                       | 5                                   | 12                                  | .0358   |
| 2B                       | 28                                  | 17                                  | .0266   |
| 3                        | 0                                   | 2                                   | .8281   |
| 4                        | 0                                   | 1                                   |         |
| Surgical margins         |                                     |                                     | .7332   |
| Negative                 | 25                                  | 24                                  | .7221   |
| Positive                 | 8                                   | 8                                   |         |
| Sex                      |                                     |                                     | .1701   |
| Female                   | 19                                  | 12                                  |         |
| Male                     | 14                                  | 20                                  |         |
| Location                 |                                     |                                     | .2935   |
| Body                     | 0                                   | 3                                   |         |
| Body tail                | 1                                   | 0                                   |         |
| Diffuse                  | 0                                   | 1                                   |         |
| Head                     | 27                                  | 24                                  |         |
| Head body                | 1                                   | 0                                   |         |
| Neck                     | 0                                   | 1                                   |         |
| Tail                     |                                     |                                     | .5944   |
| Tumor grade              |                                     |                                     | .0819   |
| Moderate                 | 18                                  | 12                                  |         |
| Moderate to poor         | 8                                   | 8                                   |         |
| Poor                     | 4                                   | 6                                   |         |
| Well                     | 2                                   | 3                                   |         |
| Well to moderate         | 1                                   | 3                                   |         |
| Histology                |                                     |                                     | .9876   |
| Ductal Adenocarcinoma    | 33                                  | 31                                  |         |
| IPMN carcinoma           | 0                                   | 1                                   |         |
| Solid pattern            |                                     |                                     | .6973   |
| Yes                      | 30                                  | 29                                  | .0428   |
| No                       | 3                                   | 3                                   |         |
| Mucinous features        |                                     |                                     | .9782   |
| Yes                      | 32                                  | 30                                  | .2057   |
| No                       | 1                                   | 2                                   |         |
| Clear cell features      |                                     |                                     | .6487   |
| Yes                      | 56                                  | 6                                   | .4867   |
| No                       | 3                                   | 0                                   |         |
| Micropapillary growth    |                                     |                                     | .9507   |
| Yes                      | 54                                  | 6                                   | .9144   |
| No                       | 5                                   | 0                                   |         |
| Features of possible concomitant IPMN |                 |                                     | .8399   |
| Yes                      | 52                                  | 6                                   | .3521   |
| No                       | 7                                   | 0                                   |         |
Table 5. Continued

| Stroma subtype | CSF-1 high | CSF-1 low | P  | Tumoral subtype | CSF-1 high | CSF-1 low | P  |
|----------------|------------|-----------|----|----------------|------------|-----------|----|
| ECM-rich       | 12         | 16        | .0769 | Basal-like     | 9          | 13        | .6025 |
| Immune-rich    | 17         | 8         |     | Classic        | 22         | 21        |     |
| NA             | 4          | 8         |     |               |            |           |     |

NOTE. Clinical variables were compared in CSF-1 high vs CSF-1 low groups (comprising tumors with higher or lower CSF-1 mRNA expression than the median, respectively), both in the stroma and in the tumor epithelial compartments, using the unpaired t test or the chi-squared test for continuous or categoric variables, respectively. Underline indicates P < .1. Boldface indicates P < .05.
AJCC, American Joint Committee on Cancer; IPMN, Intraductal Papillary Mucinous Neoplasm.

Institutes of Health, Bethesda, MD) or Qupath software (opensource software for digital pathology image) (5 fields per mouse, at least 5 mice per group, as indicated in the figure legends).

For immunofluorescence staining, the following antibodies were used: anti-mannose receptor (anti-CD206, ab64693, 1/500; Abcam), anti-cytokeratin-19 (conjugated to Alexa Fluor Fluor 647, ab192980, 1/100; Abcam), and anti-α- SMA, anti-sst1, and anti-CSF-1 as for immunohistochemistry, and then in 4',6-diamidino-2-phenylindole (1/1000, D9545; Sigma-Aldrich), plus secondary antibodies goat anti-mouse Alexa Fluor 488 (1/1000, A-11001; Invitrogen, ThermoFisher Scientific) or goat anti-rabbit Alexa Fluor 647 (1/1000, A-21244; Invitrogen). Slides were mounted in fluorescent mounting medium (S3023; Dako), and images were acquired using a Cell Observer Videomicroscope (Zeiss) or a confocal microscope (LSM 780; Carl Zeiss Microscopy, Marly Le Roi, France). Quantification was performed using ImageJ software on multiple tissue sections (5 fields per mouse, at least 5 mice per group, as indicated in the figure legends).

Blood Vessel Density and Pericyte Coverage
Tumor sections were co-immunostained by anti-MEA32 for detecting tumor vessels and anti-nueron glial 2 (NG2) antibodies for staining pericytes. Tumor vessels and pericyte coverage analysis were performed as described by Gilles et al. Briefly, tumor blood vessel density and pericyte coverage were plotted as the percentage of the MECA32 staining normalized to nuclei or the percentage of the co-localization between MECA32 and NG2 normalized to nuclei. Immunofluorescence images of tumor sections of PDA were captured by using a FV1000 IX81 laser-scanning confocal microscope (Olympus, Thorlabs, Maisons-Laffitte, France), with a 10× or 40× oil objective. Acquisitions were performed with the same settings on multiple tissue sections (5 fields per mouse, at least 5 mice per group, as indicated in the figure legends) and included negative controls for determination of background staining, which was negligible.

Flow-Cytometric Analysis
Single-cell suspensions were prepared (Tumor Dissociation Kit, 130-096-730, Miltenyi Biotec, Paris, France) and incubated with fluorophore-conjugated anti-mouse antibodies (CD45 [30-F11], CD3 [17-A2], CD4 [RM4-5], CD8 [53-6.7], CD11b [M1/70], Ly-6g [1A8]; all from BD Biosciences, Le Pont de Claix, France), CD206 (C068C2) (from BioLegend EU Amsterdam, NL), and F4/80 (BM8; from Thermofisher Scientific). Data acquisition was performed on the Fortessa LSR (BD Biosciences) and BD FacsDiva software was used for analysis, as published previously.

Western Blot
Cell lysis was performed in lysis buffer (20 mmol/L Tris, pH 7.5, 150 mmol/L NaCl, 1 mmol/L EDTA, 1% NP40, 1 mmol/L sodium orthovanadate, 1 mmol/L sodium fluoride, and a cocktail of protease inhibitors; Roche (Basel, Switzerland), and tumors were crushed with the Precellys device (Ozyme, Saint Cyr l’Ecole, France) in lysis buffer. The protein extract concentration was measured using the Protein Assay reagent (Bio-Rad). Equal amounts of proteins were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and electroblotted onto nitrocellulose membranes. Membranes were blocked (5% powdered milk in Tris-buffered saline with 0.1% Tween 20) followed by incubation with primary antibodies (anti-CSF-1 [ab99178, dilution 1/1000] and anti-sst1 antibody [ab2366, dilution 1/1000] from Abcam; anti-Akt [CST9272, dilution 1/1000] and anti-phospho-Akt [p473-Akt; CST2013, dilution 1/1000] from Cell Signalling Technologies; anti-β-actin [A5441, dilution 1/1000] is from Millipore (Merck, Fontenay-sous-Bois, France); and anti-periostin [sc 67233, dilution 1/200] from Santa Cruz (SCBT, Dallas, TX). Membranes were incubated with horseradish-peroxidase–coupled secondary antibody and treated with Pierce ECL Western Blotting Substrate (Thermofisher Scientific) before detection with the PXi imaging system (Syngene, Cambridge, UK). SUNSET assay, a nonradioactive equivalent of 35S-Met assay based on puromycin incorporation into nascent polypeptides, was used to monitor the rate of protein synthesis using an
antipuromycin antibody (12D10, dilution 1/1000; Merck Millipore).²¹

ELISA and Cytokine Arrays
CSF-1 detection in plasma was performed using the Mouse Macrophage CSF Quantikine ELISA Kit (MMC00; R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. Cytokine detection in patientCAF culture supernatant was performed using ELISA kits (macrophage-CSF, DMCO08; connective tissue growth factor, DY9190-05; THSB2 (thrombospondin-2), DTS2P20; all from R&D Systems, and biglycan ab245709; Abcam), according to the manufacturer’s instructions. Cytokine assay was performed using the Proteome Profiler Mouse XL Cytokine Array Kit (ARY028; R&D Systems) according to the manufacturer’s instructions. Membrane chemiluminescences were captured with the PXi imaging system (Syngene, Cambridge, UK). Data acquisition and quantification was performed using Quick Spots Tool software (Western Vision Software, Salt Lake City, UT).

Quantitative Real-Time PCR
Total RNA from PSCs and spleen was isolated using TRIzol (ThermoFisher Scientific). Reverse-transcription quantitative PCR reactions were performed using ReverAid H Minus Reverse Transcriptase (ThermoFisher Scientific) and SoFast EvaGreen Supermix (Bio-Rad) according to the manufacturer’s instructions and measured with the StepOne Real-Time PCR Systems (ThermoFisher Scientific). Relative quantitation values were normalized using the comparative Ct (cycle threshold) method. Primers for mouse SSTR1 (somatostatin receptor subtype 1) were as follows: forward: 5’-TGCCCTTTCTGTCATCCTCC-3’, reverse: 5’-ACGGGTCACAATTGGAACCA-3’, and for mouse housekeeping RPS16 (40S ribosomal protein S16) were as follows: forward: 5’-AATGGGCTCTAAGGTAACCA-3’, reverse: 5’-TATCCACACCCAGAAATCGCTGCT-3’.

Patient Pancreatic Cancer Tissues for Immunohistochemistry and RNAseq Analyses
Thirty-eight consecutive patients were selected from a retrospective cohort of patients with localized (non-metastatic) PDA who underwent complete surgical resection between December 2011 and January 2014 at Beaujon University Hospital (Clichy, France; Biobank registration number BB-0033-00078).²² All participants provided their informed consent before taking part. Exclusion criteria were as follows: neoadjuvant chemotherapy and/or radiotherapy, macroscopically incomplete resection (R2), tumor histology other than PDA, and insufficient tumor material available for research. Clinical and pathologic variables were collected retrospectively, including sex, age at diagnosis, tumor site, preoperative assessment of tumor extension, surgical procedure, resection margins (R0, negative; R1, positive), differentiation grade, tumor size, tumor stage, lymph node status (negative, N₀; positive, N+), LNR (ie, the ratio of lymph nodes with tumor metastasis to the total lymph nodes analyzed), TNM classification, and the presence of vascular and perineural invasion. The outcome variable was overall survival, defined as the time interval between the day of surgical resection and death (all causes) or date of last follow-up evaluation, at which point data were censored. Survival curves were estimated using the Kaplan–Meier method and compared using the log-rank test. For immunohistochemistry analyses, whole formalin-fixed paraffin-embedded (FFPE) slides were stained with each indicated antibody, after manual selection by a specialist pancreatic pathologist (J.C.) of tumoral areas on scanned slides. For αSMA (M0851; Dako) and pan-cytokeratin (MSK098-05; Zytomed, Berlin, Germany), the Precision image analysis software (Aperio, Leica Biosystems, Nanterre, France) was used for automated biomarker quantification. The activated stroma index was adapted from Erkan et al²⁰ by considering the following formula: 1 – pan-cytokeratin–stained area, as a surrogate for the collagen deposit area and calculated using the following formula: αSMA-stained surface/(1 – pan-cytokeratin–stained surface). Anti-CD8–stained (M710301; Dako) and anti-CD163–stained (NCL-CD163; Leica) tumor slides were assessed visually by 2 observers (J.C. and C.N.), to score according to the localization (peritumoral or intratumoral) and frequency of respective positive cell aggregates (low score, no intratumoral aggregate with possible low frequency of peritumoral aggregates; high score, low or high frequency of intratumoral aggregates with possible high frequency of peritumoral aggregates). For CSF-1 stain quantification in stromal or tumor areas, the calculated CSF-1 immunohistochemistry score takes into account the stain intensity and the percentage of the stained cell area. Percentages of cells expressing low, medium, and high CSF-1 stain were quantified using Definiens Tissue Studio (Imag’IN core Institut Universitaire du Cancer, Toulouse, France) in stromal or tumor compartments, as we published previously,⁴⁷ and a CSF-1 score was calculated as follows: 1 × % low CSF-1 cells + 2 × % medium CSF-1 cells + 3 × % high CSF-1 cells. Associations between the activated stroma index, CD163, or CD8 scores (high vs low, ie, higher than or less than the median, respectively), and CSF-1 immunohistochemistry scores in the stroma or in the tumor were assessed using unpaired Student t tests.

For RNAseq analyses, FFPE blocks were selected by a specialist pancreatic pathologist (J.C.). The presence of neoplastic cells was confirmed and tumor-enriched zones after examination of H&E–stained slides were macrodissected for nucleic acid extraction. For all samples, RNA was extracted using the Qiagen RNeasy FFPE kit following the manufacturer’s instructions (Qiagen, Hilden, Germany). A total of 150 ng RNA was used as starting material for the preparation of RNAseq libraries using the QuantSeq 3’ REV kit (Lexogen, Vienna, Austria). Libraries were sequenced on an Illumina (San Diego, CA) NovaSeq platform aiming for 7Millions read pairs.

Bioinformatics
For secretome analysis, GSEA was performed using the fast GSEA implementation (10.18129/B9.bioc.fgsea;
Biocorntector, open source software), using the matrix of the measurable proteins identified in CAF conditioned media (n = 9) preranked by the significance of the differential test (untreated vs SOM230-treated condition). Therefore, secretome GSEA results show enrichments, induced by SOM230, among the proteins identified in CAF conditioned media. PDX transcriptome analyses were obtained from the processed data set of the PaCaOmics clinical trial (NCT01692873). Laser-microdissected stromal and epithelial compartments of RNAseq analyses from 65 PDA patients were obtained from the gene omnibus database (GSE93326). Patient clinical data were available and correlation with stromal and tumoral CSF-1 mRNA expression was assessed. The transcriptomic data set from the Beaujon cohort RNAseq analyses on PDA FFPE tissues (n = 38 patients) was characterized according to the 6 robust transcriptomic components of the tumor and of the stroma, identified by independent component analysis in a published cohort of 309 PDA patients (ie, tumoral basal, tumoral classic, stromal activated, stromal inflammatory, stromal structured vascularized, and stromal immune), assigning a weight of each component to each FFPE sample. Associations between CSF-1 immunohistochemistry scores in the stroma or in the tumor (high vs low) and transcriptomic components were assessed using unpaired Student t tests.

Statistics
All statistical analyses were performed using GraphPad Prism 6 software (GraphPad Software, Inc, San Diego, CA). The number of animals and replicate in vitro experiments are specified in each figure legend. Results are presented as the means ± SEM. The Kaplan–Meier method was used to estimate survival rates, and significance was determined by the log-rank test. Normal distribution of variables was computed using the D’Agostino–Pearson normality test. Comparison of a continuous variable in 2 groups or more than 2 groups with normal distribution was performed using the parametric test (ie, t test or analysis of variance, respectively), and with a Bonferroni post-test for multi-parametric analyses. If the variable was not distributed normally, a nonparametric test (ie, Mann–Whitney or Kruskal–Wallis), was applied for comparison in 2 or more groups, respectively, with a Dunn post-test for multi-parametric analyses. The significance of metastasis frequency was determined by the Fisher exact test. All P values were 2-sided, and P < .05 was considered statistically significant.

All authors had access to the study data and reviewed and approved the final manuscript.

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**CRediT Authorship Contributions**

Rémi Samain, PharmD, PhD (Conceptualization: Supporting; Formal analysis: Equal; Investigation: Equal; Methodology: Equal; Writing – original draft: Supporting; Writing – review & editing: Supporting)

Alexia Brunel, MS (Formal analysis: Supporting; Investigation: Supporting; Methodology: Supporting)

Thibault Douché, MS (Investigation: Supporting; Methodology: Supporting)

Marjorie Fanjul, PhD (Methodology: Supporting)

Julia Rochotte, PhD (Formal analysis: Supporting; Investigation: Supporting; Methodology: Supporting)

Stéphanie Cassant-Sourdy, Tech (Investigation: Supporting)

Jérôme Cros, MD, PhD (Investigation: Supporting; Resources: Lead)

Cindy Neuzillet, MD, PhD (Investigation: Supporting; Methodology: Supporting)

Rémy Nicolle, PhD (Conceptualization: Supporting; Investigation: Supporting; Methodology: Supporting; Writing – review & editing: Supporting)

Yvan Martineau, PhD (Conceptualization: Supporting; Funding acquisition: Supporting; Investigation: Supporting; Methodology: Supporting; Supervision: Supporting; Writing – review & editing: Supporting)

Christine Jean, PhD (Conceptualization: Supporting; Formal analysis: Supporting; Funding acquisition: Supporting; Investigation: Supporting; Methodology: Supporting; Supervision: Equal; Writing – review & editing: Supporting)

Corinne Bousquet, VMD, PhD (Conceptualization: Lead; Formal analysis: Lead; Funding acquisition: Lead; Investigation: Supporting; Methodology: Supporting; Project administration: Lead; Supervision: Lead; Validation: Lead; Writing – original draft: Lead)

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