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New Potential Therapeutic Strategy of Pancreatic Cancer *via* Targeting the Tumor Microenvironment

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Abstract

Pancreatic cancer is one of the most lethal types of cancers, with an overall five-year survival rate of less than 5%. Radical surgical resection remains a potential cure for this refractory disease but only a small number of patients could be benefit from surgery. The interactions between pancreatic cancer cells and stromal cells induce the formation of immunosuppressive and desmoplastic tumor environment and contribute to tumor progression, chemoresistance, and immune escape. In this review, we describe majority of the acellular and cellular components of tumor microenvironment and specifically review the importance of them in TME. Additionally, we discuss the most promising therapeutic strategies for targeting those components or reshaping the pancreatic tumor stroma. Finally, current clinical trials and novel investigational agents for pancreatic cancer targeting components of the microenvironment are also briefly evaluated.

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Introduction

Pancreatic Ductal Adenocarcinoma (PDAC) is a highly aggressive human malignancy with the fourth leading cancer-related death in the United States currently. As is reliably predicted, PDAC will probably overtake the position of breast, prostate, and colon cancers to become the second leading cause of cancer death, following only lung cancer in the next decade [1]. Even though surgical resection with negative margins in combination with adjuvant therapy serves the only potentially curative therapy, approximately 70% to 80% of PDAC patients are inoperable because of local advanced or metastatic diseases [2,3]. Recently, the utilization of FOLFIRINOX or nab-paclitaxel plus gemcitabine in neoadjuvant therapy of borderline resectable pancreatic cancer and metastatic cancer is likely to improve overall survival in some patients to a limited extent [4,5]. However, 5-year survival rate in pancreatic cancer patients is lower than 10%, and considering the lack of effective treatment, there is no significant progression over the last 40 years [2].

The poor prognosis is multifactorial, not only the highly aggressive nature and complex mutational of the tumor is contributed to this refractory disease, but also the unique tumor microenvironment adds the fuel to the flame [6]. Tumor microenvironment is complex mixture of cellular and acellular components, which contains cancer-associated fibroblasts, myofibroblasts, pancreatic stellate cells, immune cells, collagen, fibronectin, extracellular matrix and multiple soluble factors in stoma [7]. Compared with other solid tumors the tumor microenvironment of pancreatic cancer is characterized by dense fibrosis, significant desmoplastic response, and abundant infiltration of immune cells [7]. The aforementioned features may pertain an essential role in the aspects of initiating malignancy, inducing immunosuppression, promoting tumor progression and metastasis, as well as delivering drug resistance. With the further exploration of constituents and functions of tumor microenvironment being crucial to the biology of PDAC, there could be potentially valuable presentation on targeting tumor microenvironment therapy and subsequent clinical developments have been attempted [8]. Hence, this review serves to delineate the current developing application of target therapy for tumor microenvironment to seek for a potentially more effective therapeutic strategy in pancreatic cancer.

Targeting tumor stroma

Hyaluronic acid (HA): Hyaluronic Acid is one of several glycosaminoglycans that comprise the ECM, abundant in the PDAC and correlated with a poor prognosis. In pancreas cancer, high HA content is present as high as 90% of PDAC tissue [9]. Along with fibrotic collagens, HA contributes to the TME component of PDAC thus leading to elevated interstitial fluid, which is known to induce chemoresistance *via* impairing delivery of systematic therapy to the tumor core. Besides HA also binds to CD44 and RHAMM cell surface receptors to activate downstream cell signaling pathways

that promote cancer cell survival, adhesion, proliferation, migration, and invasion [10]. Based on those roles, HA is considered a potential therapeutic target to remodel the tumor microenvironment and to enhance the effectiveness of systematic therapy [10,11]. Subsequent mouse studies demonstrated that PEGPH20 (a PEGylated recombinant human hyaluronidase), is functioned to normalize intra-tumor interstitial fluid pressures, relieve vascular collapse, and enhance intra-tumor chemotherapy delivery [12]. In a Phase I clinical trial, combination PEGH20 with gemcitabine-based chemotherapy improved both progression free and overall survival in advanced PDAC cases (NCT01453153). These promising results led to the randomized phase II trial, PEGPH20 in combination with gemcitabine and nab-paclitaxel for treatment of metastatic pancreatic cancer compared with gemcitabine and nab-paclitaxel alone [13-15]. In spite of a significant increase of thromboembolic events in PEGPH20 arm, the triplet arm with PEGPH20 yielded a significantly higher objective response rate (52% *vs.* 24%) and longer DFS (9.2 *vs.* 4.3 months; HR, 0.39; P=0.05) than gemcitabine and nab-paclitaxel [16]. These findings were even more evidenced in patients that had high levels of HA expression. The patients with high level of HA in tumor presented a more significant improvement in PFS (9.2 with PEGPH20 *vs.* 5.2 months, (HR, 0.45; 95% CI, 0.17 to 1.22; p=0.11) [17]. Considering this promising result, a phase III study has been initiated to select patients with high HA PDACs and anticoagulation is now administered in conjunction with PEGPH20 to precontrol the side effects of PEGH20 [18].

Focal adhesion kinase (FAK): Focal Adhesion kinase (FAK) is non-receptor tyrosine kinases which include FAK1 and PYSK2 (FAK2) is implicated in cancer migration, proliferation and survival [19]. Hyperactivated FAK in neoplastic PDAC cells contributes to fibrotic remodeling of ECM and immunosuppression of tumor microenvironment. Besides, it also functions to regulate pro-inflammatory pathway activation and to promote epithelial-mesenchymal transition [20]. In a KPC mouse model of PDAC, high level of FAK activity was observed and associated with CD8+ cytotoxic T cell infiltration, what's more, the addition of FAK inhibitor VS-4718 reduced tumor fibrosis and decreased numbers of tumor infiltrating immunosuppressive cells [21]. FAK inhibitors were also shown to enhance sensitivity of immunotherapy such as PD-1 antagonists or CTLA-4 antibodies. While no clinical trials have yet demonstrated a reliable response to single-agent FAK inhibition in PDAC [22]. Recently several trials of PDAC treatment with FAK inhibitor are active, and a phase I study of combination of FAK inhibitor and PD-1 antagonist is underway (phase I, NCT02546531).

Matrix metalloproteinase (MMPs): Matrix Metalloproteinases (MMPs) are a family of proteolytic enzymes that regulate the tumor cell invasion potential and migration [23]. Overexpression of MMPs in PDAC is responsible for tumor progression, invasion, and metastasis [24]. Preclinical study demonstrated that when stimulated the secretion and activation of MMPs by TGFβ1, there is markedly increased invasive and metastatic potential observed in cancer cells. Based on these factors, inhibition of MMPs was considered a potential therapeutic strategy to target PDAC [25,26]. Marimastat, an MMP inhibitor showed single-agent activity and safety in PDAC patients but the further therapy in combination with gemcitabine present no benefit compared to gemcitabine alone [27]. Additionally, another MMP inhibitor, tanomastat (BAY 12–956), a specific inhibitor of MMP2, -3, -9, and -13, made a similar result when compared to single-agent therapy of gemcitabine, with 1-year survival rates of 10% respectively [28]. These frustrated results urge us to further study the mechanism of MMPs in tumor invasion and migration to explore more effective therapy. *In vivo*, targeting MT1-MMP has been proposed to sensitize pancreatic tumors to gemcitabine, and attempts at developing selective MT1-MMP inhibitors have been utilized [25,29]. Recent pre-clinical study using anti-MMP9 antibody in combination with nab-paclitaxel-based for chemotherapy has been observed a potential benefit of antitumor and anti-stromal effect, besides, it was certified to promote antitumor immunity by activating T cells in another solid tumor microenvironment [30]. Other explored approaches using miRNA to regulate multiple MMPs expression could serve as an attractive therapeutic target [31]. Future efforts will be orientated on exploring more effective therapeutic strategy through targeting metalloproteinases [32].

ROCK Rho kinase inhibitor: The Rho/ ROCK signal pathway play an essential role in regulating cell shape, cell adhesion, mobility and interaction with surrounding ECM [33]. In pancreatic cancer, Rho interacts with tumor cells and tumor stroma to mediated the drug resistance mechanism. ROCK is the key mediator of RhoA activity, and ROCK is overexpressed in PDAC and exerts its effect on metastases *via* collagen remodeling in the ECM [34]. Given that following characteristics, an emerging therapeutic strategy using Rho kinase inhibitor may be potentially beneficial. Short priming with ROCK inhibitors has shown promising effects with chemotherapy in pre-clinical models. The administration of Fasudil, an RHO kinase inhibitor (priming) improved the response to gemcitabine/nab-paclitaxel chemotherapy in pancreatic cancer cell line and patient-derived tumors [35,36].

Pirfenidone: Pirfenidone is a known antifibrotic agent that mitigates the expression several mediators of fibrosis such as TGF-β and collagen [37]. In pancreatic cancer, Pirfenidone modulate PSC mediated-stromal accumulation and enhance delivery of chemotherapy [38]. Combination of pirfenidone with gemcitabine efficiently inhibits tumor growth compared to either drug independently. In preclinical studies pirfenidone has demonstrated some promising effects in combination with gemcitabine, with β-cyclodextrin MMP2 responsive liposome and with the antioxidant N-acetyl cysteine [39]. Besides, Pirfenidone could induce G0/G1 cell cycle arrest to disrupt the proliferation of pancreatic cancer cells [40]. This novel therapeutic strategy has shown potential value on targeting not only fibroblasts but also tumor cells in the tumor microenvironment, also it might be further evaluated and studied.

Targeting cellular components

Pancreatic stellate cell: PSCs are star-shaped stromal cells located at the basolateral aspect of acinar cells or the surrounding perivascular and periductal regions in the pancreas. Under homeostatic conditions, most of the PSCs stay quiescent and have the function of reserving vitamin A- rich lipid droplets, exo/endocrine secretion, and maintenance of pancreatic stromal architecture, stimulation of amylase secretion, phagocytosis, and immunity [41]. While in the PDAC tumor microenvironment, cancer cells secrete variable amounts of pro-inflammatory growth factors/cytokines, including TGF-b1, PDGF, TNF-a, and IL-1/6 and activate PSCs. Activated PSCs (aPSCs) lose cytoplasmic vitamin A- storing lipid droplets and express Alpha-Smooth Muscle Actin (α-SMA), transform into a myofibroblast-like phenotype and secrete large amounts of Extracellular Matrix (ECM) proteins, comprised of collagens, fibronectins, and laminins in periacinar regions to form desmoplastic

tissue [42,43]. In addition, the aPSCs are the most important cellular source of Cancer-Associated Fibroblasts (CAFs), and the aPSCs can also crosstalk with tumor cells and immune cells by secretion of varieties of cytokines, chemokines, Growth Factors (GFs), and exosomes to induce tumorigenesis and immunosuppression. Therefore, target therapy at PSCs could be beneficial to the treatment of pancreatic cancer [44-46].

Conventional therapeutic strategies with chemotherapy for PDAC are able to reduce desmoplasia *via* hampering the PSCs mediated tumor microenvironment remodeling, fail to gain significant survival benefits because of impaired drug delivery by the stiff stroma. In addition, some investigations indicated that therapeutic strategies aiming to deplete PSCs and myofibroblast contribute to immunosuppression, enhanced tumor hypoxia, EMT program and enhanced aggressiveness of PDAC [47]. Hedgehog (Hh) signaling pathway is currently known to activate PSCs *via* paracrine effects and to regulate stromal abundance. Therapeutic effects using hedgehog inhibitor, IPI-926 and gemcitabine as a combination therapy had been explored, however, the outcome turn out to more aggressive disease and conversed survival benefit [48,49]. Other attempts *via* stroma-depleting therapeutic strategies contribute to similar results to IPI-926 [50]. Considering that dissatisfaction, more emphasis should be focus on inhibiting the activation of quiescent PSCs or reversing aPSCs or CAFs into quiescent PSCs. Aforementioned aPSCs are often in deficiency of cytoplasmic vitamin A -storing lipid droplets, and patients with PDAC also lack of vitamin A and D. *In vitro*, All-Trans Retinoic Acid (ATRA) were observed to reverse aPSCs into quiescent PSCs and contribute to inhibition on aPSC migration and collagen synthesis [51]. Current phase Ib study is under way investigating ATRA along with gemcitabine and nab-paclitaxel in PDAC [52]. Besides, aPSCs express high levels of the Vitamin D Receptor and its related pathway is essential factor to regulate the conversion of activated state. In contrast to gemcitabine treatment alone, treatment using VDR ligand in combination with gemcitabine result in promising survival benefit and shrunken tumor volume in KPC mice model [53-54]. Recent study revealed that miRNAs are potentially valuable for targeting PSCs [55]. miRNA-29 were maintained in decreased level in aPSCs and restore miR-29 expression reduced stroma accumulation and tumor growth. Other miRNA sequence, for example, miRNA-21, miRNA-199a and miRNA-214 were found overexpressed in CAFs and aPSCs may be future explored for target therapy [56,57].

Cancer-associated fibroblast: Cancer-Associated Fibroblasts (CAFs) are activated fibroblasts which originated from resident fibroblasts and Pancreatic Stellate Cells (PSCs), the recruitment and differentiation of bone marrow-derived MSCs and Epithelial-to-Mesenchymal Transition (EMT) [58-60]. Pioneer study with single-cell analysis has defined three major types of fibroblasts in pancreatic cancer, inflammatory CAFs (iCAFs), myofibroblastic CAFs (myCAFs), and antigen-presenting CAFs (apCAFs) [61,62]. Indirect evidence suggests that different subpopulations of CAFs may function in diversity [63,64]. In tumor microenvironment activated CAFs can secrete abundant collagens, fibronectin, Matrix Metalloproteinases (MMPs), growth factors and cytokines, and act a critical role in tumor growth, formation of stem cell niches, immunosuppression, metastasis, chemoresistance and ECM remodeling [65]. Retrospective analyses have shown that high levels of α-SMA or FAPα expression on fibroblasts in tumor stroma are evidently related with overall survival [65-68]. Depletion of α-SMA+ fibroblasts result in decreased survival but disruption of tumor-promoting desmoplasia with depletion of FAP-expressing fibroblasts displayed reduced tumor growth and obtained a remarkable achievement in survival [69]. Until now, the mechanism of fibroblasts activation is still obscure. TGF-β, secreted by both cancer cells and stromal cells, is able to activate CAFs; however, TGF-β alone is not sufficient to endow fibroblasts with all the features of activated CAFs *in vitro* [70]. Future therapeutic strategies could pay attention to the signal pathway of fibroblasts activation and the function of different subtypes of CAFs to seek for the therapeutic potentiality.

Tumor associated macrophages (TAMs): Tumor Associated Macrophages (TAMs) reside within the TME in PDAC and are thought to serve as a potential therapeutic target in several solid tumors. In fact, Tumor-Associated Macrophages (TAMs) have been identified as one of the most abundant immune infiltrated cells in PDAC [71,72]. M1-like polarized macrophages, capable of pro-inflammatory reaction and phagocytosis, are recognized as anti-tumor macrophages. And different from M1-like macrophages, M2-like polarized macrophages contribute to development of an immunosuppressive microenvironment and progression of tumor growth by secreting anti-inflammatory cytokines, proteases and growth factors [73]. Therapeutic strategies aiming to reduce the rate of M2 polarization TAMs and reprogram M2 TAMs into an effective anti-tumor activity have started to emerge. CL2-CCR2 pathway has been shown to play a critical role in recruitment of TAMs [74,75]. In an orthotropic model of mouse PDAC, CCR2 blockade using a small molecule inhibitor PF-04136309 depleted inflammatory monocytes and macrophages in the TME and decreased tumor growth and metastatic potential [76]. In a phase I ongoing study, combined with PF-04136309, Gemcitabine plus Nab-Paclitaxel severed a result that 4-month disease control rate was 70%. Besides, low dose irradiation in xenotransplant mouse models reprogrammed M2-like to M1-like macrophages, which resulted in effector T-cell recruitment [77]. Modulated macrophage polarization towards M1-like phenotype by adjuvant gemcitabine-based chemotherapy could reach a similar result [78]. CD40, as a macrophage cell surface marker, can inhibit cytotoxic functions. Using CD40 agonist antibodies induce high expression of M1 markers contribute to a significant antitumor effect and enhancement of chemotherapy [79]. It is noteworthy that the crosstalk between macrophages, tumor cells, and stromal cells have a complicated influential on the polarization and function of macrophages [80]. In addition, previous study revealed that several signal pathways are engaged in macrophage polarization such as JNK, PI3K/Akt, Notch and JAK/STAT signals pathway [81]. Further investigation of novel agents targeting TAM in human pancreatic cancer is warranted to with the hope of improving the outcome of this fatal disease.

Myeloid-derived suppressor cells (MDSCs): Myeloid-Derived Suppressor Cells are a population of cells defined by their immature state, myeloid origin and capacity to suppress the immune response [82]. They are strongly immunosuppressive by their ability to inhibit T-cell proliferation, IFN-γ production, effector T-cell function and macrophage conversion into M2 phenotypes, and to favor Treg generation through the secretion of ROS, Arg1, and iNOS [83]. Besides, upregulation of the Programmed Death-Ligand 1 (PD-L1) on MDSCs is responsible for immunosuppression in the TME by rendering the inactivation of lymphocytes [84]. In PDAC, MDSCs promote tumor growth by VEGF and MMP9 secretions. Pancreatic cancer cells can induce mobilization of MDSCs out of the bone marrow

and into systemic circulation before they get recruited into the TME, what's more, high concentration of MDSCs in the peripheral blood is associated with poor prognosis in PDAC [85]. Thus, the inhibition of MDSCs is a potential therapeutic target in PDAC. Targeted depletion of Granulocytic MDSC (Gr-MDSC) activates an endogenous anti-tumor T cell response to enhance apoptosis of tumor cells, indicating perhaps Gr-MDSC may be a promising therapeutic target to PDAC [86]. Inhibition of CXCR2 signaling blocked the recruitment of granulocytic MDSCs to the tumor site and significantly enhanced the efficacy of PD-1 blockade [84,87].

Tumor infiltrating T cells: Lymphocytes that infiltrate the tumor are called Tumor-Infiltrated Lymphocytes (TILs). Upon examination of established PDAC there is an abundance of CD4+ T cells and a paucity of CD8+ T cells in the TME [88,89]. There are sufficient studies illustrating that CD4+ T cells play an immunosuppressive role in the tumor microenvironment and the presumed mechanism may be over blocking the anti-tumor immune response of CD8+ T cells [90]. The ratio for Th2/Th1 tumor infiltrating lymphocytes was an independent predictive marker for survival after surgery in patients with (stage IIB/III) pancreatic cancer [91]. T-cell dependent antitumor immunity is thought to be in part *via* activation of the TNF receptor superfamily member CD40 [92]. Thus, through an indirect effect, CD40 activating monoclonal antibodies activate CD8+ T cells and potentially can reverse the immunosuppressive environment observed in pancreas cancer. In a phase I study, CP-8970,893, a CD40 agonist antibody, was given in combination with gemcitabine in patients with treatment naive unresectable PDAC. Four of the 22 enrolled patients experienced a partial response, which demonstrated the potential of CD40 agonists as a treatment modality in pancreas cancer. In addition, there is a clinical study investigating the efficiency of gemcitabine/nab-paclitaxel chemotherapy combined with CD40 agonistic monoclonal antibody and anti-PD-1 monoclonal antibody in pancreatic cancer patients [93].

Immune checkpoint: The PDAC tumor microenvironment has been characterized by few tumor cells with a robust infiltrate of immune cells, usually dominated by Myeloid Derived Suppressor Cells (MDSCs), Tumor-Associated Macrophages (TAMs) and neutrophils, with TILs present but in smaller number [94,95]. The massive accumulation of infiltrated MDSCs, TAMs, TILs develop the immunosuppressive tumor microenvironment by hampering effective T cell cytotoxicity and inducing immune escape of tumor cell. In this process, the immune checkpoint plays an essential role [96]. Targeting immune checkpoints is that blockade of the inhibitors harnesses the endogenous anti-tumor response of the immune system to combat the disease. PD-1 binds to PD-1 ligands PD-L1 and PD-L2, resulting in activation and suppression of T-cell activity and allowing for immune escape of tumor cells in the TME [97]. Emerging immunotherapy strategies with the application of PD-1/ PD-L1 checkpoint inhibitor are investigated. As a monotherapy, PD-1/ PD-L1 inhibitor didn't reach a satisfied disease response in clinical trials for PDAC [97,98], but current combinatorial strategies with chemoradiotherapy and other immunotherapy like cancer vaccine, targeting immune cell or stroma therapy provides a favorable data of survival and safety. Advanced study demonstrated that checkpoint inhibitors are likely to enhance other immunotherapy by disruption of crosstalk between tumor cells and immune cells [99-103]. CTLA-4 is another protein receptor that acts as an immune checkpoint expressed in PDAC, in pre-clinical animal trials CTLA-4 inhibitor showed promise with prolonged survival, but failed to gain survival benefit in phase II trial [104]. Up to now, although targeting immune checkpoint has been successful in other tumor types, clinical trials in PDAC did not demonstrate significant disease response and survival advantages [105]. In future, we should focus more energy on the exploration of other potential immune checkpoint and multiple targeted approaches for PDAC.

Conclusion

The treatment of pancreatic adenocarcinoma remains a challenge because of limited effective treatment options in spite of several targets in PDAC. Recent fundamental advances in the understanding of tumor microenvironment have created opportunities for the development of more effective and personalized approaches for pancreatic cancer patients. Nevertheless, results from some preclinical and clinical studies targeting the tumor microenvironment were still controversial, suggesting a comprehensive and in-depth research on the basic science of the tumor microenvironment. Future work focusing on combinatorial approach of targeting tumor cells, targeting TME and immunotherapy is needed to explore the more ideal treatment strategy for PDAC.

References

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics. CA Cancer J Clin. 2019;69(1):7-34.
2. Neoptolemos JP, Palmer DH, Ghaneh P, Psarelli EE, Valle JW, Halloran CM, et al. Comparison of Adjuvant Gemcitabine and Capecitabine with Gemcitabine Monotherapy in Patients with Resected Pancreatic Cancer(ESPAC-4): AMulticentre, Open-label, Randomised, Phase 3 Trial. Lancet. 2017;389(10073):1011-24.
3. Daniel DV, Thomas E, Francis PA, Gabriela C, Jeffrey I, Malcolm M, et al. Increased Survival in Pancreatic Cancer with nab-Paclitaxel plus Gemcitabine. N Engl J Med. 2013;369(18):1691-703.
4. Conroy T, Françoise D, Ychou M. FOLFIRINOX versus Gemcitabine for Metastatic Pancreatic Cancer. N Eng J Med. 2011;364(19):1817-25.
5. Tahara J, Shimizu K, Otsuka N, Akao J, Takayama Y, Tokushige K. Gemcitabine Plus Nab-paclitaxel *vs.* FOLFIRINOX for Patients with Advanced Pancreatic Cancer. Cancer Chemother Pharmacol. 2018;82(2):245-50.
6. Neesse A, Algül H, Tuveson DA, Gress TM. Stromal Biology and Therapy in Pancreatic Cancer: A changing paradigm. Gut. 2011;64(9):1476-84.
7. Dougan SK. The Pancreatic Cancer Microenvironment. Cancer J. 2017;23(6):321-5.
8. Rucki AA, Zheng L. Pancreatic Cancer Stroma: Understanding Biology Leads to New Therapeutic Strategies. World J Gastroenterol. 2014;20(9):2237-46.
9. Toole BP, Slomiany MG. Hyaluronan: AConstitutive Regulator of Chemoresistance and Malignancy in Cancer Cells. Semin Cancer Biol. 2008;18(4):244-50.
10. Provenzano PP, Cuevas C, Chang AE, Goel VK, Von Hoff DD, Hingorani SR. Enzymatic Targeting of the Stroma Ablates Physical Barriers to Treatment of Pancreatic Ductal Adenocarcinoma. Cancer Cell. 2012;21(3):418-29.
11. Thompson CB, Shepard HM, O'Connor PM. Enzymatic Depletion of Tumor Hyaluronan Induces Antitumor Responses in Preclinical Animal Models. Mol Cancer Ther. 2010;9(11):3052-64.
12. Jacobetz MA, Chan DS, Neesse A. Hyaluronan Impairs Vascular Function and Drug Delivery in a Mouse Model of Pancreatic Cancer. Gut. 2013,62(1):112-20.
13. Hingorani SR, HarrisWP, Beck JT. Phase Ib Study of PEGylated

Recombinant Human Hyaluronidase and Gemcitabine in Patients with Advanced Pancreatic Cancer. Clin Cancer Res. 2016;22(12):2848-54.

14. Strimpakos AS, Saif MW. Update on Phase I Studies in Advanced Pancreatic Adenocarcinoma. Hunting in Darkness? Jop. 2013;14(14):354-8.

15. Hingorani SR, Harris WP, Hendifar AE, Bullock AJ, Wu XW, Huang Y, et al. High Response Rate and PFS with PEGPH20 Added to Nabpaclitaxel/Gemcitabine in Stage IV Previously Untreated Pancreatic Cancer Patients with High-HA Tumors: Interim Results of a Randomized Phase II Study. J Clin Oncol. 2015;33(Supp 15):4006.

16. Hingorani S, Bullock A, Harris W. PD-006 Final Analysis of Stage 1 Data from a Randomized Phase II Study of PEGPH20 Plus Nab-Paclitaxel/Gemcitabine in Stage IV Previously Untreated Pancreatic Cancer Patients, Utilizing Ventana Companion Diagnostic Assay. Ann Oncol. 2016;27(Supp2):ii103.

17. Hingorani SR, Zheng L, Bullock AJ, Seery TE, Harris WP, Sigal DS, et al. HALO 202: Randomized Phase II Study of PEGPH20 Plus Nab-Paclitaxel/Gemcitabine versus Nab-Paclitaxel/Gemcitabine in Patients with Untreated, Metastatic Pancreatic Ductal Adenocarcinoma. J Clin Oncol. 2018;36(4):359-66.

18. Bahn JD, Skipper J, Garcia A, Jiang P, Osgood R, Symons R, et al. Abstract 2501: PEGylated human recombinant hyaluronidase (PEGPH20) enhances radiotherapy in hyaluronan-rich solid tumors. Tumor Biology. 2011;71(8 Suppl):2501-2501.

19. Stokes JB, Adair SJ, Slack-Davis JK, Walters DM, Tilghman RW, Hershey ED, et al. Inhibition of focal adhesion kinase by PF-562,271 inhibits the growth and metastasis of pancreatic cancer concomitant with altering the tumor microenvironment. Mol Cancer Ther. 2011;10(11):2135-45.

20. Miyazaki T, Kato H, Nakajima M, Sohda M, Fukai Y, Masuda N, et al. FAK overexpression is correlated with tumour invasiveness and lymph node metastasis in oesophageal squamous cell carcinoma. Br J Cancer. 2003;89(1):140-5.

21. Jiang H, Hegde S, Knolhoff BL, Zhu Y, Herndon JM, Meyer MA, et al. Targeting focal adhesion kinase renders pancreatic cancers responsive to checkpoint immunotherapy. Nat Med. 2016;22(8):851-60.

22. Infante JR, Camidge DR, Mileshkin LR, Chen EX, Hicks RJ, Rischin D, et al. Safety, pharmacokinetic, and pharmacodynamic phase I dose-escalation trial of PF-00562271, an inhibitor of focal adhesion kinase, in advanced solid tumors. J Clin Oncol. 2012;30(13):1527-33.

23. Bramhall SR, Neoptolemos JP, Stamp GW, Lemoine NR. Imbalance of expression of matrix metalloproteinases (MMPs) and tissue inhibitors of the matrix metalloproteinases (TIMPs) in human pancreatic carcinoma. J Pathol. 1997;182(3):347-55.

24. Bloomston M, Zervos EE, Rosemurgy AS 2nd. Matrix metalloproteinases and their role in pancreatic cancer: a review of preclinical studies and clinical trials. Ann Surg Oncol. 2002;9(7):668-74.

25. Krantz SB, Shields MA, Dangi-Garimella S, Cheon EC, Barron MR, Hwang RF, et al. MT1-MMP cooperates with Kras(G12D) to promote pancreatic fibrosis through increased TGF-β signaling. Mol Cancer Res. 2011;9(10):1294-304

26. Roy R, Yang J, Moses MA. Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer. J Clin Oncol. 2009;27(31):5287-97.

27. Bramhall SR, Rosemurgy A, Brown PD, Bowry C, Buckels JA. Marimastat as first-line therapy for patients with unresectable pancreatic cancer: a randomized trial. J Clin Oncol. 2001;19(15):3447-55.

28. Moore MJ, Hamm J, Dancey J, Eisenberg PD, Dagenais M, Fields A, et al. Comparison of gemcitabine versus the matrix metalloproteinase inhibitor BAY 12-9566 in patients with advanced or metastatic adenocarcinoma of the pancreas: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol. 2003;21(17):3296-302.

29. Dangi-Garimella S, Krantz SB, Barron MR, Shields MA, Heiferman MJ, Grippo PJ, et al. Three-dimensional collagen I promotes gemcitabine resistance in pancreatic cancer through MT1-MMP-mediated expression of HMGA2. Cancer Res. 2011;71(3):1019-28.

30. Lauer-Fields JL, Whitehead JK, Li S, Hammer RP, Brew K, Fields GB. Selective modulation of matrix metalloproteinase 9 (MMP-9) functions *via*. exosite inhibition. J Biol Chem. 2008;283(29):20087-95.

31. Xu Q, Li P, Chen X, Zong L, Jiang Z. miR-221/222 induces pancreatic cancer progression through the regulation of matrix metalloproteinases. Oncotarget. 2015;6(16):14153-64.

32. Mroczko B, Lukaszewicz-Zając M, Gryko M, Kędra B, Szmitkowski M. Clinical significance of serum levels of matrix metalloproteinase 2 (MMP-2) and its tissue inhibitor (TIMP-2) in gastric cancer. Folia Histochem Cytobiol. 2011;49(1):125-31.

33. Schofield AV, Bernard O. Rho-associated coiled-coil kinase (ROCK) signaling and disease. Crit Rev Biochem Mol Biol. 2013;48(4):301-16.

34. Rath N, Morton JP, Julian L, Helbig L, Kadir S, McGhee EJ. ROCK signaling promotes collagen remodeling to facilitate invasive pancreatic ductal adenocarcinoma tumor cell growth. EMBO Mol Med. 2017;9(2):198-218.

35. Vennin C, Chin VT, Warren SC, Lucas M, Herrmann D, Magenau A, et al. Transient tissue priming *via* ROCK inhibition uncouples pancreatic cancer progression, sensitivity to chemotherapy, and metastasis. Sci Transl Med. 2017;9(384). pii: eaai8504.

36. Whatcott CJ, Ng S, Barrett MT, Hostetter G, Von Hoff DD, Han H. Inhibition of ROCK1 kinase modulates both tumor cells and stromal fibroblasts in pancreatic cancer. PLoS One. 2017;12(8):e0183871.

37. Di Sario A, Bendia E, SvegliatiBaroni G, Ridolfi F, Casini A, Ceni E, et al. Effect of pirfenidone on rat hepatic stellate cell proliferation and collagen production. J Hepatol. 2002;37(5):584-91.

38. Kozono S, Ohuchida K, Eguchi D, Ikenaga N, Fujiwara K, Cui L, et al. Pirfenidone inhibits pancreatic cancer desmoplasia by regulating stellate cells. Cancer Res. 2013;73(7):2345-56.

39. Ji T, Li S, Zhang Y, Lang J, Ding Y, Zhao X, et al. An MMP-2 Responsive Liposome Integrating Antifibrosis and Chemotherapeutic Drugs for Enhanced Drug Perfusion and Efficacy in Pancreatic Cancer. ACS Appl Mater Interfaces. 2016;8(5):3438-45.

40. Usugi E, Ishii K, Hirokawa Y, Kanayama K, Matsuda C, Uchida K, et al. Antifibrotic Agent Pirfenidone Suppresses Proliferation of Human Pancreatic Cancer Cells by Inducing G0/G1 Cell Cycle Arrest. Pharmacology. 2019;103(5-6):250-6.

41. Apte MV, Wilson JS, Lugea A, Pandol SJ. A starring role for stellate cells in the pancreatic cancer microenvironment. Gastroenterology. 2013;144(6):1210-9.

42. Allam A, Thomsen AR, Gothwal M, Saha D, Maurer J, Brunner TB. Pancreatic stellate cells in pancreatic cancer: In focus. Pancreatology. 2017;17(4):514-522.

43. Omary MB, Lugea A, Lowe AW, Pandol SJ. The pancreatic stellate cell: a star on the rise in pancreatic diseases. J Clin Invest. 2007;117(1):50-9.

44. Fu Y, Liu S, Zeng S, Shen H. The critical roles of activated stellate cells-mediated paracrine signaling, metabolism and onco-immunology in pancreatic ductal adenocarcinoma. Mol Cancer. 2018;17(1):62.

45. Mace TA, Bloomston M, Lesinski GB. Pancreatic cancer-associated stellate cells: A viable target for reducing immunosuppression in the tumor microenvironment. Oncoimmunology. 2013;2(7):e24891.

46. Zhang A, Qian Y. Cancer-associated fibroblasts promote M2 polarization of macrophages in pancreatic ductal adenocarcinoma. Cancer Med. 2017;6(2):463-70.

47. Olive KP, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, et al. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science. 2009;324(5933):1457-61.

48. Rhim AD, Oberstein PE, Thomas DH, Mirek ET, Palermo CF, Sastra SA, et al. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. Cancer Cell. 2014;25(6):735-47.

49. Jacobetz MA, Chan DS, Neesse A, Bapiro TE, Cook N, Frese KK, et al. Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer. Gut. 2013;62(1):112-20.

50. Özdemir BC, Pentcheva-Hoang T, Carstens JL, Zheng X, Wu CC, Simpson TR, et al. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. Cancer Cell. 2014;25(6):719-34.

51. Jaster R, Hilgendorf I, Fitzner B, Brock P, Sparmann G, Emmrich J, et al. Regulation of pancreatic stellate cell function *in vitro*: biological and molecular effects of all-trans retinoic acid. Biochem Pharmacol. 2003;66(4):633-41.

52. Kocher H, David JP, Sara A, Mousa K, Bernard N, Vasia P, et al. STAR_PAC: A Phase 1B study repurposing ATRA as stromal targeting agent along with gemcitabine and nab Paclitaxel for pancreatic cancer. Pancreatology. 2016;16(3):S4-S5.

53. Wolpin BM, Kimmie Ng, Bao Y, Kraft P, Stampfer MJ, Michaud DS, et al. Plasma 25-Hydroxyvitamin D and Risk of Pancreatic Cancer. Cancer Epidemiol Biomarkers Prev. 2012;21:82–9

54. Sherman MH, Yu RT, Engle DD, Ding N, Atkins AR, Tiriac H, et al. Vitamin D receptor-mediated stromal reprogramming suppresses pancreatitis and enhances pancreatic cancer therapy. Cell. 2014;159(1):80-93.

55. Kota J, Hancock J, Kwon J, Korc M. Pancreatic cancer: Stroma and its current and emerging targeted therapies. Cancer Lett. 2017;391:38-49.

56. Praneeth RK, Linda B, Prakash J. MicroRNA-199a and -214 as potential therapeutic targets in pancreatic stellate cells in pancreatic tumor. Oncotarget. 2016;7(13):16396–408.

57. Kadera BE, Li L, Toste PA, Wu N, Adams C, Dawson DW, et al. MicroRNA-21 in pancreatic ductal adenocarcinoma tumor-associated fibroblasts promotes metastasis. PLoS One. 2013;8(8):e71978.

58. Kalluri R. The biology and function of fibroblasts in cancer. Nat Rev Cancer. 2016;16(9):582-98.

59. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell. 2009;139(5):871-90.

60. Bergfeld SA, DeClerck YA. Bone marrow-derived mesenchymal stem cells and the tumor microenvironment. Cancer Metastasis Rev. 2010;29(2):249-61.

61. Su S, Chen J, Yao H, Liu J, Yu S, Lao L, et al. CD10+GPR77+ Cancer-Associated Fibroblasts Promote Cancer Formation and Chemoresistance by Sustaining Cancer Stemness. Cell. 2018;172(4):841-56.

62. Elyada E, Bolisetty M, Laise P, Flynn WF, Courtois ET, Burkhart RA, et al. Cross-Species Single-Cell Analysis of Pancreatic Ductal Adenocarcinoma Reveals Antigen-Presenting Cancer-Associated Fibroblasts. Cancer Discov. 2019;9(8):1102-23.

63. Ireland LV, Mielgo A. Macrophages and Fibroblasts, Key Players in Cancer Chemoresistance. Front Cell Dev Biol. 2018;6:131.

64. Biffi G, Tuveson DA. Deciphering cancer fibroblasts. J Exp Med. 2018;215(12):2967-8.

65. Kawase T, Yasui Y, Nishina S, Hara Y, Yanatori I, Tomiyama Y, et al. Fibroblast activation protein-α-expressing fibroblasts promote the progression of pancreatic ductal adenocarcinoma. BMC Gastroenterol. 2015;15:109.

66. Fujita H, Ohuchida K, Mizumoto K, Nakata K, Yu J, Kayashima T, et al. alpha-Smooth Muscle Actin Expressing Stroma Promotes an Aggressive Tumor Biology in Pancreatic Ductal Adenocarcinoma. Pancreas. 2010;39(8):1254-62.

67. Shi M, Yu DH, Chen Y, Zhao CY, Zhang J, Liu QH, et al. Expression of fibroblast activation protein in human pancreatic adenocarcinoma and its clinicopathological significance. World J Gastroenterol. 2012;18(8):840-6

68. Cremasco V, Astarita JL, Grauel AL, Keerthivasan S, MacIsaac K, Woodruff MC, et al. FAP Delineates Heterogeneous and Functionally Divergent Stromal Cells in Immune-Excluded Breast Tumors. Cancer Immunol Res. 2018;6(12):1472-85.

69. Lo A, Wang LS, Scholler J, Monslow J, Avery D, Newick K, et al. Tumor-Promoting Desmoplasia Is Disrupted by Depleting FAP-Expressing Stromal Cells. Cancer Res. 2015;75(14):2800-10.

70. Stylianou A, Gkretsi V, Stylianopoulos T. Transforming growth factor-β modulates pancreatic cancer associated fibroblasts cell shape, stiffness and invasion. Biochim Biophys Acta Gen Subj. 2018;1862(7):1537-46.

71. Habtezion A, Edderkaoui M, Pandolb SJ. Macrophages and pancreatic ductal adenocarcinoma. Cancer Lett. 2016;381(1):211–6

72. Zhu Y, Herndon JM, Sojka DK, Kim KW, Knolhoff BL, Zuo, et al. Tissue-Resident Macrophages in Pancreatic Ductal Adenocarcinoma Originate from Embryonic Hematopoiesis and Promote Tumor Progression. Immunity. 2017;47(2):323-38.

73. Ngambenjawong C, Gustafson HH, Pun SH. Progress in tumor-associated macrophage (TAM)-targeted therapeutics. Adv Drug Deliv Rev. 2017;114:206-21.

74. Wolf MJ, Hoos A, Bauer J, Boettcher S, Knust M, Weber A, et al. Endothelial CCR2 signaling induced by colon carcinoma cells enables extravasation via the JAK2-Stat5 and p38MAPK pathway. Cancer Cell. 2012;22(1):91-105.

75. Bonapace L, Coissieux MM, Wyckoff J, Mertz KD, Varga Z, Junt T, et al. Cessation of CCL2 inhibition accelerates breast cancer metastasis by promoting angiogenesis. Nature. 2014;515(7525):130-3.

76. Sanford DE, Belt BA, Panni RZ, Mayer A, Deshpande AD, Carpenter D, et al. Inflammatory monocyte mobilization decreases patient survival in pancreatic cancer: a role for targeting the CCL2/CCR2 axis. Clin Cancer Res. 2013;19(13):3404-15.

77. Klug F, Prakash H, Huber PE, Seibel T, Bender N, Halama N, et al. Low-dose irradiation programs macrophage differentiation to an iNOS /M1 phenotype that orchestrates effective T cell immunotherapy. Cancer Cell. 2013;24(5):589-602.

78. Caro DG, Cortese N, Castino GF, Grizzi F, Gavazzi F, Ridolfi C, et al. Dual prognostic significance of tumour-associated macrophages in human pancreatic adenocarcinoma treated or untreated with chemotherapy. Gut. 2016;65(10):1710-20.

79. Beatty GL, Torigian DA, Chiorean EG, Saboury B, Brothers A, Alavi A, et al. A phase I study of an agonist CD40 monoclonal antibody (CP-870,893) in combination with gemcitabine in patients with advanced pancreatic ductal adenocarcinoma. Clin Cancer Res. 2013;19(22):6286-95.

80. Zhan HX, Zhou B, Cheng YG, Xu JW, Wang L, Zhang GY, et al. Crosstalk between stromal cells and cancer cells in pancreatic cancer: New insights into stromal biology. Cancer Lett. 2017;392:83-93.

81. Zhou D, Huang C, Lin Z, Zhan S, Kong L, Fang C, et al. Macrophage polarization and function with emphasis on the evolving roles of coordinated regulation of cellular signaling pathways. Cell Signal. 2014;26(2):192-7.

82. Talmadge JE, Gabrilovich DI. History of myeloid-derived suppressor cells. Nat Rev Cancer. 2013;13(10):739-52.

83. Pergamo M, Miller G. Myeloid-derived suppressor cells and their role in pancreatic cancer. Cancer Gene Ther. 2017;24(3):100-105.

84. Pinton L, Solito S, Damuzzo V, Francescato S, Pozzuoli A, Berizzi A, et al. Activated T cells sustain myeloid-derived suppressor cell-mediated immune suppression. Oncotarget. 2016;7(2):1168-84.

85. Diaz-Montero CM, Salem ML, Nishimura MI, Garrett-Mayer E, Cole DJ, Montero AJ. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. Cancer Immunol Immunother. 2009;58(1):49-59.

86. Stromnes IM, Brockenbrough JS, Izeradjene K, Carlson MA, Cuevas C, Simmons RM, et al. Targeted depletion of an MDSC subset unmasks pancreatic ductal adenocarcinoma to adaptive immunity. Gut. 2014;63(11):1769-81.

87. Steele CW, Karim SA, Leach JDG, Bailey P, Upstill-Goddard R, Rishi L, et al. CXCR2 Inhibition Profoundly Suppresses Metastases and Augments Immunotherapy in Pancreatic Ductal Adenocarcinoma. Cancer Cell. 2016;29(6):832-45.

88. Liu L, Zhao G, Wu W, Rong Y, Jin D, Wang D, et al. Low intratumoral regulatory T cells and high peritumoral CD8(+) T cells relate to long-term survival in patients with pancreatic ductal adenocarcinoma after pancreatectomy. Cancer Immunol Immunother. 2016;65(1):73-82.

89. Shibuya KC, Goel VK, Xiong W, Sham JG, Pollack SM, Leahy AM, et al. Pancreatic ductal adenocarcinoma contains an effector and regulatory immune cell infiltrate that is altered by multimodal neoadjuvant treatment. PLoS One. 2014;9(5):e96565.

90. McAllister F, Bailey JM, Alsina J, Nirschl CJ, Sharma R, Fan H, et al. Oncogenic Kras activates a hematopoietic-to-epithelial IL-17 signaling axis in preinvasive pancreatic neoplasia. Cancer Cell. 2014;25(5):621-37.

91. Monte DL, Reni M, Tassi E, Clavenna D, Papa I, Recalde H, et al. Intratumor T helper type 2 cell infiltrate correlates with cancer-associated fibroblast thymic stromal lymphopoietin production and reduced survival in pancreatic cancer. J Exp Med. 2011;208(3):469-78.

92. Diehl L, den Boer AT, Schoenberger SP, van der Voort EI, Schumacher TN, Melief CJ, et al. CD40 activation *in vivo* overcomes peptide-induced peripheral cytotoxic T-lymphocyte tolerance and augments anti-tumor vaccine efficacy. Nat Med. 1999;5(7):774-9.

93. Beatty GL, Torigian DA, Chiorean EG, Saboury B, Brothers A, Alavi A, et al. A phase I study of an agonist CD40 monoclonal antibody (CP-870,893) in combination with gemcitabine in patients with advanced pancreatic ductal adenocarcinoma. Clin Cancer Res. 201319(22):6286-95.

94. Bailey P, Chang DK, Forget MA, Lucas FA, Alvarez HA, Haymaker C, et al. Exploiting the neoantigen landscape for immunotherapy of pancreatic ductal adenocarcinoma. Sci Rep. 2016;6:35848.

95. Clark CE, Beatty GL, Vonderheide RH. Immunosurveillance of pancreatic adenocarcinoma: insights from genetically engineered mouse models of cancer. Cancer Lett. 2009;279(1):1-7.

96. Johansson H, Andersson R, Bauden M, Hammes S, Holdenrieder S, Ansari D. Immune checkpoint therapy for pancreatic cancer. World J Gastroenterol. 2016;22(43):9457–76.

97. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366(26):2455-65.

98. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252-64.

99. Nomi T, Sho M, Akahori T, Hamada K, Kubo A, Kanehiro H, et al. Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. Clin Cancer Res. 2017;13(7):2151-7.

100. Beatty GL, Torigian DA, Chiorean EG, Saboury B, Brothers A, Alavi A, et al. A Phase I Study of an Agonist CD40 Monoclonal Antibody (CP-870,893) in Combination with Gemcitabine in Patients with Advanced Pancreatic Ductal Adenocarcinoma. Clin Cancer Res. 2013;19:6286–95.

101. Gordon SR, Maute RL. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. Nature. 2017;545(7655):495-9.

102. Kleponis J, Skelton R, Zheng L. Fueling the engine and releasing the break: combinational therapy of cancer vaccines and immune checkpoint inhibitors. Cancer Biol Med. 2015;12(3):201-8.

103. Soares KC, Rucki AA, Wu AA, Olino K, Xiao Q, Chai Y, et al. PD-1/PD-L1 blockade together with vaccine therapy facilitates effector T-cell infiltration into pancreatic tumors. J Immunother. 2015;38(1):1-11.

104. Winograd R, Byrne KT, Evans RA, Odorizzi PM, Meyer AR, Bajor DL, et al. Induction of T-cell Immunity Overcomes Complete Resistance to PD-1 and CTLA-4 Blockade and Improves Survival in Pancreatic Carcinoma. Cancer Immunol Res. 2015;3(4):399-411.

105. Royal RE, Levy C, Turner K, Mathur A, Hughes M, Kammula US, et al. Phase II trial of single agent Ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. J Immunother. 2010;33(8):828-33.