Review Article

Shifting paradigm of developing biologics for the treatment of pancreatic adenocarcinoma

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Abstract: Pancreatic adenocarcinoma is still widely considered as a deadly disease even though there are substantial therapeutic developments in the past decade. Using combinational chemotherapy regimens, represented by gemcitabine plus nab-paclitaxel and FOLFIRINOX, was able to improve overall survival in patients with advanced disease to a limited extent. It has been a challenge to develop targeted therapies that are focused on the neoplasm cells of pancreatic adenocarcinoma. Recently, targeting the stroma and immune compartments of pancreatic adenocarcinoma has shown promising results. The paradigm of biologics drug development therefore has been shifted by extending to these exciting areas. Although some of the preclinical and clinical researches in targeting the tumor microenvironment of pancreatic adenocarcinoma have shown promising results, others have resulted in controversial findings. Both comprehensive and in-depth researches on the basic science of the tumor microenvironment of pancreatic adenocarcinoma are thus warranted for the development of effective biologics that target the tumor microenvironment. Moreover, an ideal treatment for pancreatic adenocarcinoma shall be a combination of targeting both neoplastic cells and the tumor microenvironment.

Keywords: Pancreatic adenocarcinoma; chemotherapy; targeted therapy; immunotherapy

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The landscape of the pancreatic ductal adenocarcinoma (PDAC) genome is notable for four frequently mutated genes (*KRAS*, *TP53*, *p16/CDKN2A*, *DPC4/SMAD4*). So far, there is no effective targeted therapy for these four driver mutations. It is known over 90 percent of PDACs harbor a *KRAS* gene mutation. Mutational active oncogenic KRAS engages the PI3K-PDK1-AKT pathway to drive cancer initiation, progression and maintenance. Additionally, activated KRAS signals through the MAPK pathway via RAF-MEK1/2-ERK1/2. However, all attempts to target KRAS directly have failed in the clinic and KRAS is still widely considered to be undruggable (1). Epidermal growth factors receptor (EGFR) is a direct upstream of KRAS (2). It was reported that drugs targeting EGFR will lose efficiency if KRAS is activated (3,4). Although erlotinib, a tyrosine kinase inhibitor of EGFR, is approved by FDA in combination with gemcitabine for the first-line treatment of advanced PDA, it offers minimal clinical benefit (5), likely due to the high prevalence of the *KRAS* mutation. For the same reason, it is not surprising to see the negative results of the phase III study of testing the combination of gemcitabine and anti-EGFR antibodies. Not until we have more effective therapeutic agents to target the four frequently mutated genes in PDAC, developing targeted

therapies that are solely focused on the neoplasm cells of PDAC is unlikely to be successful.

With a better understanding of complex stromal constituents and desmoplastic stromal reaction being crucial to the biology of PDAC, the paradigm of drug development in PDAC has been shifted to focuses on both neoplastic cells and their tumor microenvironment. It first became evident that targeting the stromal components showed a benefit in preclinical mouse models of PDAC (6). Subsequently, clinical developments have been attempted in targeting the following stromal components.

Sonic hedgehog (Shh)

Beneficial effect of Shh pathway inhibition has been demonstrated in the treatment of basal cell carcinoma. Vismodegib (GDC-0449), a small-molecule inhibitor of the hedgehog pathway, showed 30–43% response rate in advanced basal-cell carcinoma and was FDA approved in 2012 (7). Inhibition of Shh in preclinical mouse models showed better gemcitabine delivery, stromal depletion and increased vascularization of PDAC tumors (8). Based on those intriguing results, a few different Shh inhibitors have recently been tested in clinical trials in combination with gemcitabine or FOLFIRINOX (the combination of 5-FU, irinotecan, and oxaliplatin) for metastatic PDACs (9). IPI-926 (Smo inhibitor) given in combination with gemcitabine showed partial responses in three out of nine patients, however the combination of IPI-926 and gemcitabine did not yield any survival benefit comparing to gemcitabine alone (10). Vismodegib is currently being tested in combination with gemcitabine and nab-paclitaxel (human-albumin-bound paclitaxel, ABRAXANE) in a single arm phase II clinical trial in patients with previously untreated metastatic PDA to evaluate disease free survival (DFS) (NCT01088815). Overall, the results from targeting the stroma of PDAC through Shh inhibition have been disappointing. Studies in the mouse models of PDACs are ongoing in an attempt to reveal the underlying mechanisms of the failure in targeting Shh. Given the complexity of the signaling in the stroma, simultaneous modulation of other stromal signaling is perceived as the next step of drug development.

Hyaluronic acid

Hyaluronic acid is another important stromal target in PDAC. It has been demonstrated in mouse models of PDAC, enzymatic degradation of HA resulted in increased gemcitabine tumor cytotoxicity due to relief of vascular collapse (11). Those prove-of-principle experiments led to the development of PEGPH20 (pegylated recombinant human hyaluronidase—an enzyme that degrades HA). In phase Ib clinical trial, PEGPH20 given with gemcitabine in patients with stage IV PDAC resulted partial response in 43% of patients and stable disease in additional 30% patients. Specifically for those patients whose PDACs expressed high level of HA, partial response was seen in 64% patients (12). In the randomized phase II clinical trial, PEGPH20 is given in combination with gemcitabine and nab-paclitaxel, in the subgroup of patients whose PDACs express a high level of HA, gemcitabine and nab-paclitaxel in combination 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. A trend toward improved overall survival was also observed (12 *vs.* 9 months; HR, 0.62). (ASCO-GI 2016 abstract 439). In light of this result, a phase III study has been initiated to select patients with high HA PDACs for comparing gemcitabine and nab-paclitaxel with PEGPH20 *vs.* placebo (NCT02715804).

JAK/STAT

The activation of JAK/STAT pathway is very well known in hematologic malignancies. The JAK family of kinases includes JAK1, JAK2, JAK3 and TYK2. JAK kinases are activated through tyrosine phosphorylation of the cytoplasmic domains of cytokine receptors upon cytokine binding. Activation of JAK promotes recruitment of the transcription factors STAT to the receptor complex, leading to the nuclear translocation of STAT and transcription of genes that regulate cell proliferation, differentiation and apoptosis (13,14).

In addition to the well-studied somatic point mutation in *JAK2* gene in myeloproliferative neoplasms, over activation of JAK/STAT pathway with or without *JAK2* mutation has been reported in some solid tumors and inflammatory conditions (15,16). Emerging preclinical evidence showed activation of JAK/STAT pathway and related inflammatory process promote development and progression of pancreatic cancer (17,18). In particular, STAT3 plays a critical role and is required for KRAS induced pancreatic tumorigenesis (19-22).

Proinflammatory cytokine activity is associated with weight loss, hypermetabolism, anorexia, cachexia, and it is also strongly implicated in the development and progression of

malignancies (23-25). Among the many inflammatory markers studied to date, serum C-reactive protein (CRP) is the most well-characterized inflammation marker in cancer with variety of clinical scenarios including pancreatic cancer (26). The systemic inflammation-based Glasgow Prognostic Score (GPS), the combination of CRP and albumin, is clearly implicated in the prognosis of patients with cancer (27).

The role of ruxolitinib, a potent JAK1/2 inhibitor, in myeloproliferative neoplasms is very well established. Its efficacy in treatment of pancreatic cancer was tested in combination with capecitabine in a randomized, double-blind, phase II clinical trial (the RECAP trial). Patients with metastatic pancreatic cancer who failed gemcitabine based chemotherapy were randomized 1:1 to ruxolitinib plus capecitabine or placebo plus capecitabine. Even though the trial didn't reach its primary end point for overall survival (HR, 0.79; P=0.25), the pre-specified subgroup analysis of patients with inflammation, defined by CRP greater than the study population median (13 mg/L), overall survival was significantly greater with ruxolitinib compared with placebo (HR, 0.47; P=0.011) (28). Based on these data, two randomized, double-blind, phase III trials (the JANUS 1 and JANUS 2) were undertaken to test ruxolitinib or placebo in combination with capecitabine in patients with advanced or metastatic pancreatic cancer who have failed or are intolerant to first-line chemotherapy (NCT02117479, NCT02119663). However JANUS 1 and JANUS 2 trials were both discontinued after a planned interim analysis of JANUS 1 demonstrated that ruxolitinib plus capecitabine did not show a sufficient level of efficacy to warrant continuation. A more specific biomarker may be needed for selecting patients that may benefit from the treatment with JAK inhibitors.

Transforming growth factor β (TGF-β)

TGF-β expression is increased in PDAC and associated with poor prognosis (29,30). The sources of TGF-β appear to be predominant in the tumor microenvironment. TGF-β is a multifunctional cytokine, including inhibiting cell growth through nuclear SMAD3, activating vascular endothelial growth factor A (VEGF-A) to promote angiogenesis and metastases, and driving a fibrous reaction in the stroma (31). Trabedersen, an antisense molecule against TGF-β2, was tested in a phase I/II study for stage III/IV PDAC, malignant melanoma and metastatic colorectal cancer patients and showed a median overall survival of 13.4 months (32). A humanized monoclonal antibodies against TGF-β, fresolimumab, showed antitumor activity in a phase I study in patients with advanced malignant melanoma and renal cell carcinoma (33). Galunisertib, a small molecule inhibitor of TGF-βR1 serine/threonine kinase, also showed potential antitumor activity in glioma, PDAC and lung cancer (34). As TGF-β is a central molecule mediating multiple immunosuppressive signals, it is more intriguing to target TGF-β for the enhancement of antitumor immune response by combining TGF-β inhibitors with immunotherapy (35).

Immune compartments of the tumor microenvironment

Several approaches to immunotherapy for PDAC have shown promise in early clinical trials. The goal of immunotherapy in PDAC has been focused on inducing tumor infiltration, activation of effector cells (i.e., $CD8^+$ T cells) and consequent $CD8^+$ T cell dependent tumor lysis. Multiple clinical trials demonstrated that enhanced response of interferon-secreting mesothelin-specific $CD8^+$ T cells in peripheral lymphocytes correlated with better survival in patients with resected or metastatic PDAC who received lethally irradiated allogeneic GM-CSF secreting whole cell vaccine (GVAX) (36-38). A trend of improvement in overall survival in heavily treated metastatic PDAC patients was observed in a pilot study testing the combination of GVAX and ipilimumab (an anti-CTLA-4 therapeutic antibody) comparing to ipilimumab alone; it thus supported the role of CTLA-4 blockade in enhancing anti-tumor response of GVAX (39). However, it is still not entirely clear how vaccine-based immunotherapy activates anti-tumor effector cells within the tumor microenvironment, and identification of new targets in tumor microenvironment may help the development of immune modulatory therapies (40).

CD40, a potential immune modulatory target in tumor microenvironment, is a costimulatory molecule found on antigen presenting cells (APCs) that is required for their activation by $CD4^+$ helper cells. Only activated APCs can in turn activate naive $CD8^+$ T cells into cytotoxic effector cells. It was demonstrated in some studies that CD40 activating antibody can effectively stimulate APCs in the absence of $CD4^+$ helper cells, which then can successfully prime and activate $CD8^+$ T cells (41). Those preclinical studies led to development of activating CD40 antibodies, which have been tested in clinical trials. Agonist CD40 monoclonal antibody was shown to induce clinical responses in combination with gemcitabine in patients with

surgically incurable PDAC; in addition, in the same study, it demonstrated the antitumor activity of agonist CD40 mAb is T cell-independent, tumoricidal cells were CD40 activated macrophages and not $CD8^+$ T cells as originally expected (42). An agonist CD40 monoclonal antibody (CP-870,893) in combination with gemcitabine was tested in a phase I study in chemotherapy-naive patients with advanced PDAC. The data is promising with four out of 22 patients achieved a partial response (43).

More recently, CCL2/CCR2 chemokine signaling axis has been shown to be a promising target in treatment of PDAC. CCL2/CCR2 facilitates the recruitment of inflammatory monocytes and metastasis-associated macrophages in tumor microenvironment which are crucial for tumor immune evasion, treatment resistance and disease progression (44,45). Human pancreatic cancer produces CCL2, and immunosuppressive $CCR2^+$ macrophages infiltrate these tumors. Patients with tumors that exhibit high CCL2 expression/low CD8 T-cell infiltrate have significantly decreased survival (46). It was demonstrated in preclinical study that targeting CCR2 improves chemotherapeutic efficacy, inhibits metastasis, and increases antitumor T-cell responses (47). PF-04136309, an oral small-molecule CCR2 inhibitor, in combination with FOLFIRINOX chemotherapy (oxaliplatin and irinotecan plus leucovorin and fluorouracil) was tested in previously untreated patients with borderline resectable and locally advanced pancreatic cancer in a phase Ib trial. It demonstrated higher objective tumor response in comparison to patients who received FOLFIRINOX alone (48). A phase Ib/II study of PF-04136309 in combination with gemcitabine plus nab-paclitaxel in first-line metastatic pancreatic cancer patients was recently initiated (NCT02732938).

Other targets in the shifted paradigm of drug development

Notch signaling in cancer stem cells

Cancer stem cells are thought to play an important role in the recurrence and metastasis of PDACs, thus, have become a target for the drug development for PDAC. A growing body of evidence suggests that aberrant Notch pathway activation has been implicated in the initiation and progression of different malignancies including pancreatic cancer. Notch pathway components were found to be upregulated in pancreatic cancer stem cells. Activation of Notch signaling contributes to the acquisition of epithelia-mesenchymal transition (EMT) phenotype, and maintaining the cancer stem cell population, therefore increased chemoresistance (49-52). Notch signaling pathway has substantial crosstalk with other signaling pathways that play a significant role in cancer, including PI3K/Akt pathway.

Two main strategies were developed to target the Notch pathway. The inhibitors of the gamma secretase are the first Notch-targeting drugs. Gamma secretase frees up the Notch intracellular domain and allows the subsequent activation of the downstream signaling. A number of preclinical studies revealed gamma secretase inhibitors were effective in inducing apoptosis, tumor regression and controlling metastatic dissemination (53,54). Multiple gamma secretase inhibitors (MK-0752, RO4929097, BMS-906024, PF-03084014) are developed and currently are being evaluated in phase I and I/II clinical trials in metastatic disease including pancreatic cancer. Phase I/II study of PF-03084014 in combination with gemcitabine and nab-paclitaxel in patients with previously untreated metastatic pancreatic adenocarcinoma is currently undergoing (NCT02109445). Another phase II study of RO4929097 in patients with previously treated metastatic pancreatic adenocarcinoma is already completed its first stage, however the development of this drug has been discontinued (55).

Unlike gamma secretase inhibitors which result in pan-Notch inhibition, monoclonal antibody OMP-59R5 (tarextumab) selectively inhibits Notch2/3, and its antitumor activity was characterized by a dual mechanism of action in both tumor and stromal/vascular cells in xenograft experiments (56). Final results of phase Ib of OMP-59R5 in combination with nab-paclitaxel and gemcitabine in patients with untreated metastatic pancreatic cancer was presented at 2015 Gastrointestinal Cancer Symposium. Encouraging anti-tumor activity was observed. A total of 40 patients received treatment at 7 dose levels. Ten patients achieved partial response; stable disease was observed in 17 patients. The main dose limiting toxicity was diarrhea (ASCO-GI 2015 abstract 278). It is currently being examined in phase II ALPINE trial.

Targeting poly(ADP-ribose) polymerase (PARP) and DNA repair mechanism for familial pancreatic cancer

Additional focus of drug development is given toward the genetic defects that cause the familial pancreatic cancer. Studies indicate that about ten percent of patients with pancreatic cancer have a known genetic alteration

that predisposes them to the disease. Germline mutations in *BRCA1* and especially *BRCA2* are associated with an increased risk of pancreatic cancer. About one percent of non-*BRCA1/BRCA2* deficient familial breast cancer are contributed by germline defects in *PALB2* (partner and localizer of BRCA2) gene. The PALB2 protein binds with BRCA2 protein and stabilizes it in the nucleus; the BRCA2/PALB2 complex is part of the Fanconi anemia DNA repair pathway that acts on double-stranded DNA repair.

BRCA1/BRCA2 encodes proteins critical for homologous recombination-mediated DNA repair which mediates DNA double strand breaks (DSBs). In the absence of functional BRCA1/BRCA2 or PALB2, DSBs are repaired by the error-prone non-homologous end joining pathway which leads to genetic instability.

Tumors with mutations in the BRCA genes are vulnerable to specific DNA-damaging agents and DNA repair inhibitors. Those are the platinum-based chemotherapy agents and the newer class of drugs known as PARP inhibitors. Platinum induces inter-strand DNA cross-link; PARP inhibitors work through several mechanism: inhibition of base excision repair and trapping of PARP which leading to the induction of double-stranded breaks after stalling and collapse of the DNA replication forks (57-59).

Tumor responses and progression-free survival benefit with PARP inhibitor olaparib in breast and ovarian cancers associated with germline *BRCA1/2* mutations were demonstrated in multiple clinical trials (60-63). The clinical benefits of using platinum and PARP inhibitors in patients with pancreatic cancer and *BRCA1/BRCA2* mutation have been demonstrated in a few retrospective studies. One study included 71 patients with PDAC and *BRCA1* (n=21), *BRCA2* (n=49) or both (n=1). Median OS for patients with stage III/IV disease was 12 months (95% CI: 6–15 months), and superior OS was observed for patients with stage III/IV treated with platinum *vs.* those treated with non-platinum chemotherapies (22 *vs.* 9 months; P=0.039) (64,65). Early phase clinical trials showed some promising results. Olaparib as monotherapy in patients with advanced cancer and a germline *BRCA1/2* mutation was tested in a phase II study. A total of 62 patients with pancreatic cancer who had prior gemcitabine treatment were included. The reported tumor response rate was 12.9%, stable disease was observed in 35% patients (66). Large scale prospective trials are still awaited to confirm the clinical benefits of PARP inhibitors in PDACs with *BRCA1/BRCA2* mutation.

Prospective

While the results of targeting the driver mutations in PDACs are disappointing, our understanding of the tumor microenvironment of PDACs has advanced substantially in the last decade. Targeting the stroma and immune compartments of PDACs has shown promising results. The paradigm of biologics drug development thus has been extended to an exciting area. 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 of pancreatic adenocarcinoma are warranted. On another hand, targeting a single aspect of PDACs is unlikely successful. In the future, when the difficulty in targeting tumor cells has also been overcome, the ideal treatment strategy for PDAC shall target tumor cells and the tumor microenvironment simultaneously.

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Footnote

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