Combination Therapies and Drug Delivery Platforms in Combating Pancreatic Cancer

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Abbreviations: PC – Pancreatic cancer; PDAC – Pancreatic ductal adenocarcinoma; GEM –Gemcitabine; ECM – Extracellular matrix; PFS – Progression free survival; OS – Overall survival; RR – Response rate; 5-FU – 5-Fluorouracil; PCSCs – Pancreatic cancer stem cells

Abstract

Pancreatic ductal adenocarcinoma (PDAC), the fourth leading cause of cancer-related death in the United States, is highly aggressive and resistant to both chemo- and radiotherapy. It remains one of the most difficult-to-treat cancers, not only due to its unique pathobiological features such as stroma-rich desmoplastic tumors surrounded by hypovascular and hypoperfused vessels limiting the transport of therapeutic agents, but also to problematic early detection, which renders most treatment options largely ineffective resulting in extensive metastasis. To elevate therapeutic effectiveness of treatments and overt their toxicity, significant enthusiasm was generated to exploit new strategies for combating PDAC. Combination therapy targeting different barriers to mitigate delivery issues, reduce tumor recurrence and metastasis has demonstrated optimal outcomes in patients' survival and quality of life, providing possible approaches to overcome therapeutic challenges. This manuscript aims to provide an overview of currently explored multi-modal therapies using either conventional therapy or nanomedicines along with rationale, up-to-date progress, as well as the key challenges that must be overcome. Understanding the future directions of the field may assist in the successful development of novel treatment strategies for enhancing therapeutic efficacy in PDAC.

Key words

Pancreatic cancer, Pancreatic ductal adenocarcinoma, Therapeutic strategies, Combination therapies, Nanomedicine, Desmoplastic stromal barriers, Cancer stem cells

Introduction

Pancreatic cancer (PC) remains a treatment-refractory malignancy with a median survival of 5-6 months (Rahib et al., 2014). PCs are split into two main groups – endocrine and exocrine tumors. Pancreatic ductal adenocarcinoma (PDAC) is an exocrine tumor, representing over 90% of all pancreatic malignancies (Pelosi et al., 2017). Although PDAC accounts for only about 2% of all cancer cases, it is the fourth leading cause of death due to cancer in the United States. It is estimated that ~ 45,750 deaths from this disease would be reported in 2019 (American Cancer Society 2019). PDAC is associated with a very poor prognosis, for which mortality closely parallels incidence. The five-year survival rate has only improved marginally over the past decades and remains as low as 6%, of which perhaps the most important is that the majority of PDAC cases are diagnosed at late stages with widespread metastases (Hall et al., 2018). Although the evolution of PDAC starts from its earliest non-malignant precursor lesions, most patients with PDAC are asymptomatic until the disease develops to an advanced stage. The retroperitoneal position of the pancreas, the absences of sensitive and non-invasive biomarkers represent additional hurdles to the imaging, screening and early detection of PDAC (Kaur et al., 2012). Although surgical resection is considered as the only potentially curative treatment for PDAC, less than 20% of patients are suitable candidates for this procedure as the disease is far too advanced when diagnosed and thus inoperable (Adamska et al,. 2017). PDAC remains one of the most difficult-to-treat cancers, owing to its aggressive nature, complex tumor microenvironment, and intrinsic resistance to chemotherapeutics, which renders most treatment options mostly ineffective. Since the 1990s, single-agent gemcitabine, a

nucleoside analogue of deoxycytidine that blocks DNA replication and several forms of DNA repair, has been the standard of care for patients with advanced PDAC. For the subsequent years, many therapies were investigated to improve chemotherapeutic strategies for PDAC but the rate of successful clinical trials was relatively low (Hall et al,. 2018). To date, only two systemic therapies demonstrated the improved outcomes of the treatment as compared with gemcitabine alone, at the expense of increased adverse effects (Conroy et al,. 2011). Thus, new more effective and less toxic therapeutic strategies targeting both cancer cells and the tumor microenvironment are urgently needed for combating PDAC. In this context, nanotechnology-based drug delivery platforms can offer the possibilities to achieve efficient tumor targeting, lower drug-related toxicities and improve clinical outcome (Meng et al,. 2018). Overall, this review aims to provide a synopsis of currently explored multi-modal therapies using either conventional therapy or nanomedicines to treat PDAC, and discuss new challenges presented along with further considerations, and the crucial roles of interdisciplinary approaches.

Pathophysiology, diagnosis and staging of PDAC

PDAC follows a pathway of progression from normal ductal epithelium, to duct lesions, to invasive ductal adenocarcinoma, and it takes approximately 17 years for PDAC to progress from the tumor-initiating cell to the development of metastatic disease (Yachida et al., 2010). This process is characterized by multiple genetic alterations that trigger the tumor progression cascade, such as activation of oncogenes, of which mutationally activated *KRAS* plays an utmost vital role in tumor initiation and maintenance during the whole PDAC progression through regulation of cell division, differentiation and apoptosis (Eser et al,. 2014; Korc, 2010). On the other hand, the frequent inactivation of tumor suppressor genes, including *p16INK4A/CDKN2A*, *TP53* and *DPC4/SMAD4*, are also contribute to the deregulation of cell cycle and biological aggressiveness of PDAC (Zhang et al., 2016). *CDKN2A* gene encodes two different proteins, p16 and p14ARF, that are both cell-cycle regulators. Loss or promoter methylation of the CDKN2A gene markedly promotes cell proliferation and tumor progression. The *TP53*, a cellular stress sensor, is mutated in about 70% of human PDAC (Morton et al,. 2010). Its inactivation leads to uncontrolled cell growth and increased cell survival, and in combination with activated KRAS was shown to drive the genomic instability and tumor metastatic capacity (Bardeesy et al,. 2006). The loss or loss-of-function of the DPC4/*SMAD4* tumor suppression gene is another common genetic alteration that results in disruption of TGF-β pathway, occurs relatively late in pancreatic carcinogenesis and was found to be associated with poor prognosis in PDAC patients. In addition to the “driver mutations” in these four genes, high-frequency alterations in Wnt signalling, chromatin remodelling, Hedgehog signalling, DNA repair

and cell cycle processes were also observed (Witkiewicz et al,. 2015). By histological studies and clinical observations, it is postulated that before the final formation of invasive cancer, there is a stepwise progression of precursor lesions such as intraductal papillary mucinous neoplasms (IPMNs), mucinous cystic neoplasms (MCNs) (Hruban et al., 2004), and pancreatic intraepithelial neoplasia (PanIN), a most common and well-defined precursor to PDAC (Yonezawa et al., 2008). The progression of PanINs lesions follows a pathway from no dysplasia, to moderate dysplasia, to high-grade dysplasia (carcinoma in situ), to invasive carcinoma, accompanied by increased frequency of multiple genetic alterations (Koorstra et al., 2008; Distler et al., 2014; Muniraj et al., 2013; Kamisawa et al., 2016). Amongst different types of cancers, PC is recognized as the utmost devastating and difficult-to-treat cancer. There are a number of key features that make it a particularly challenging disease. PC is characterized by significant genomic heterogeneity: recent extensive genetic studies showed an average of 63 genetic aberrations across 12 functional pathways in the majority of PCs (Jones et al., 2008; The Cancer Genome Atlas Research Network 2017). Importantly, the pathway components that may be altered in a specific tumor vary widely which make developing tailored therapies targeting specific genes quite challenging. Tumor stroma is another defining hallmark of PDAC, which is considered as one of the most stroma-rich cancers. Dense fibrotic stroma occupies the majority of the tumor mass and consists of extracellular matrix (ECM) components and non-neoplastic cells including fibroblastic, vascular and immune cells. The excessive deposition of ECM generates high interstitial fluid pressures that compress blood vessels and causes hypoperfusion, hypovascularity, and hypoxia. This desmoplastic hypovascular tumor microenvironment is now

recognized to promote tumor growth, facilitates its invasive and metastatic potential, immunosuppression, and impairs the delivery of chemotherapeutics (Xie et al., 2015; Kaur et al., 2013).

Early and accurate diagnosis of PDAC is crucial for oncologists to determine an effective and timely treatment options for patients. Currently, there are no validated early detection strategies for PDAC, even for high-risk patients. PDAC detection and staging is usually based on a combination of imaging techniques (e.g., computed tomography, transabdominal ultrasound, and magnetic resonance imaging), tumor markers (e.g., carbohydrate antigen 19-9, carcinoembryonic antigen, and osteopontin), clinical presentations (e.g., progressive weight loss, anorexia, and abdominal pain), and the “gold standard” diagnostic tool - biopsy. Staging assessment is based on the extent of invasion into the pancreas and surrounding tissue (T), presence or absence of spread to lymph nodes (N), and presence or absence of metastasis (M). The tumor-node-metastasis (TNM) staging system serves as a clinically useful tool for prognosis, surveillance, and treatment planning as well as risk stratification in clinical trials for patients with PDAC (Zhang et al., 2016).

Therapeutic strategies

Surgical resection with chemotherapy (usually adjuvant) remains the preferable and the only potentially curative approach in PDAC clinical management. Sadly, due to the aggressive nature of the disease, late prognosis and early metastasis, patients suffering from locally advanced or metastatic PDAC at presentation are usually no longer

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candidates for surgical removal of the tumor (Manji et al., 2017). As a result, the primary goal of clinical management for these patients is to control the disease and improve the quality of life. Gemcitabine (GEM) has been accepted as the standard first-line therapy for patients having advanced PDAC. There is no alternative monotherapeutic regimen being proved to be more beneficial compared to GEM in terms of progression-free and overall survival (PFS and OS) (Conroy et al., 2016). However, due to the drawbacks of GEM (instability in plasma leading to extremely short half-life, inefficient cell uptake and complex intracellular metabolism), and fast growth of intrinsic and acquired chemo-resistance during the treatment, it is urgently calling for new and disruptive strategies for PDAC (Spadi et al., 2016; Cloyd et al., 2017). Combination therapy is emerging at this point with superior cost-to-efficacy value and overall outcomes.

Combination therapies

Combination therapy has become the major means to combat cancer thanks to its primary advantages of increased efficacy without or with minimal addictive toxicities at equal or reduced administrating doses, which is recommended for patients for most of the cases if available. Ideal combination therapy should address the following aspects – 1) maximization of therapeutic efficacy of each single drug of the combination; 2) minimization of intrinsic and acquired cross-resistance of drugs possibly occurring in the treatment; 3) diminishing overlapping adverse effects for better tolerability. The drug combination with distinct molecular mechanisms of action exhibits various remarkable improvements in treatment outcomes, ultimately leading to more promising patient compliance. Regarding PDAC, as aforementioned, GEM is the only approved first-line

monotherapy in PDAC, but unfortunately, still delivering unsatisfactory therapeutic outcomes in prolonging PFS and OS of patients with locally advanced and metastatic PDAC (Hidalgo et al., 2015). As a result, most of the combination regimens tested are centered on GEM. Multiple combination therapies composed of GEM and different cytotoxic and biologic agents have underwent clinical evaluation for the patients with various stages of PDAC. The gold standards for evaluating the efficacy of these treatments are PFS and OS (Table 1).

Generally, the GEM-based combination therapies include previously approved or well-known chemotherapeutic regimens being used for PDAC (Teague et al., 2015) such as Capecitabine, Oxaliplatin, Irinotecan, Paclitaxel, or molecular targeting agents like Erlotinib (EGFRi) (Seicean et al., 2015; Chiorean et al., 2015), Sunitinib (VEGFRi), pimasertib (MEKi), Bevacizumab (VEGF), Vismodegib (HSPi), etc. Amongst the GEM-based molecular targeting combination therapies tested in clinical trials, the only one received Food and Drug Administration (FDA) approval is Erlotinib + GEM combination, with improved PFS and OS (even though merely 2-weeks improvement in OS) (Wang et al., 2015). Combining platinum-based agents with GEM seems to be not so efficient as that with 5-fluorouracil (5-FU), demonstrating no improvement in primary measures of the trials. As a result, platinum-based agents are mostly utilized in combination c with 5-FU. Despite encouraging outcomes of other GEM-based combination therapies in early phases studies including response rate (RR), adverse effects, tolerability, most of Phase III trials failed in improving the survival benefits and quality of life, with PFS and OS still limited to 5-9 months for locally advanced or metastatic PDAC (Aprile et al., 2017). No statistically significant progresses were achieved throughout the clinical trials

compared to GEM monotherapy whereas some were even inferior to GEM monotherapy. For instance, rigosertib, a small-molecule RAS mimetic and inhibitor of the PI3K and PLK1 pathways, showed a promising synergistic activity with GEM in preclinical patient-derived xenograft models of PDAC. However, the combination of rigosertib and GEM did not improve the treatment outcome in patients with metastatic pancreatic cancer in a Phase II/III trial (NCT01360853). Median OS was 6.1 months for rigosertib + GEM combination versus 6.4 months for GEM (O'Neil et al., 2015). It was suggested that the lack of clinical activity for rigosertib in KRAS mutant PDAC patients could be linked to the inherent heterogeneity of the disease.

Rapid growth of resistance to GEM-based regimens and frequent relapse vastly accelerate the development of non-GEM based combination therapies as alternatives for PDAC patients who are refractory to GEM-containing regimens. There is proved to be low cross-resistance between GEM and 5-FU in spite they are both nucleoside analogues. Alternatively, GEM-based therapies can also be given to those previously treated with fluoropyrimidine-based therapies. Amongst them, the most impressive and successful one is FOLFIRINOX, a multidrug regimen consisting of Folinic acid, 5-FU, Irinotecan, and Oxaliplatin. Before that, extensive partial combinations of FOLFIRINOX were tested in the clinic, and three most representative regimens are FOLFOX (5-FU, Folinic Acid, and Oxaliplatin), FOLFIRI (5-FU, Folinic Acid, and Irinotecan) and XELOX (Capecitabine, oral prodrug of 5-FU, and Oxaliplatin), catalyzing the emergence of FOLFIRINOX (Vaccaro et al., 2011). The superiority of FOLFIRINOX over GEM was recognized in all efficacy parameters, including OS (11.1 vs. 6.8 months), PFS (6.4 vs. 3.3 months), and one-year survival rate (48.4% vs. 20.6%). Unfortunately, FOLFIRINOX

treatment-related side effects also are severe, including fatigue, bone marrow suppression with 45.7% grade 3 or 4 neutropenia, 12.7% diarrhea, and 9.0% sensory neuropathy and lead to the termination of treatment in a one third of patients due to poor tolerability issues. On the other hand, the quality of life measures strongly supports FOLFIRINOX for patients at the late stage with definitively less decrease in the health status (Khushman et al., 2015; Rombouts et al., 2016). Consequently, FOLFIRINOX is reserved as a preferred first-line therapy for patients with locally advanced or metastatic PDAC when they have good performance status. In some cases, it is also recommended (Jutric et al., 2017) for disease down staging or can be considered as neoadjuvant regimen for patients with borderline resectable tumors (Paniccia et al., 2014; Hackert et al., 2016; Godhi et al., 2017). In hopes for being beneficial for those unqualified patients, regimens with dose reduction of the chemotherapeutics, referred to as modified FOLFIRINOX (mFOLFIRINOX) (Marsh Rde et al., 2015), are widely implemented in the clinic and demonstrated comparable results (PFS and OS are 6.1 months and 10.2 months, respectively) with manageable adverse effects. The recent PRODIGE 24/CCTG PA.6 multicenter, randomized Phase III clinical trial (NCT01526135) also assessed the benefit of mFOLFIRINOX compared to GEM monotherapy in adjuvant setting for patients with resected PDAC. Based on reported data, median disease-free survival was 21.6 months for mFOLFIRINOX and 12.8 months for GEM. However, grade 3/4 toxicities were reported for 75.5% of patients receiving mFOLFIRINOX compared to 51.5% of patients receiving GEM (Conroy et al., 2018; Lee et al., 2016). To further extend the potential of this promising regimen, some innovative combinations with FOLFIRINOX as a chemotherapeutic platform are

underway in early phase clinical trials (Chiorean et al., 2016), including molecular targeting therapy (IPI-926, NCT01383538) (Ko et al,. 2016a), immunoradiation therapy (Nivolumab with stereotactic body radiation, NCT03563248), redox metabolism therapy (CPI-613, NCT03699319, NCT01835041), vaccination therapy (Ipilimumab, NCT01896869) and tumor-associated macrophage targeting therapy (PF-04136309, NCT01413022).

Despite the success, conventional combination therapies in the treatment of PDAC confer a small advantage over single agent therapy and survival times of PDAC patients remain unsatisfactory. Chemotherapy regimens are inevitably facing challenges such as poor bioavailability and intrinsic toxicity, compromising their efficacy and further utilization. As such, the new strategies that will allow better delivery of chemotherapeutic agents to the tumor while decreasing systemic toxicity are urgently needed. To this end, one of the promising options is an implementation of nanothechnology-based therapeutic approaches in PDAC. Indeed, the unique characteristics of nanocarriers such as their nanoscale sizes, high surface-to-volume ratios, high loading capacity and favorable drug release profiles make them suitable for delivering chemotherapeutic drugs to the target tumor tissue (Shi et al., 2017; Au et al., 2016). Physicochemical characteristics of nanocarriers can be readily adjusted to facilitate the delivery of a variety of therapeutic agents including small molecular drugs, biomacromolecules, and inorganic nanoparticles. These nanomedicines can be surface-functionalized to present targeting ligands to a receptor of interest to home them at the desired site. Combining drugs in one delivery carrier is another advantageous strategy for controlling the pharmacokinetics and co-delivery of the desired drug ratio *in vivo*, and

a variety of nanoscale carriers have been investigated in terms of their ability to deliver multiple drugs (Zhang et al., 2016; Hu et al., 2016). Several nanomedicines have been approved for clinical use for cancer treatment, including PDAC, and many others demonstrate great promise in clinical development.

Nanomedicines in PDAC treatment protocols

Currently, two nanotechnology-based therapeutics, albumin-bound paclitaxel nanoparticles, Abraxane® or nab-paclitaxel, and liposomal formulation of irinotecan, Onivyde™, have been approved for PDAC treatment in combinations with other chemotherapeutic agents (Zhang et al., 2016; Shi et al., 2017). Taxanes are useful components in the systemic treatment of many cancers but unfortunately, it exhibits very few noticeable benefits as a single or combination therapies in PDAC until the emergence of nab-paclitaxel. The encapsulation of paclitaxel into the human serum albumin-based drug delivery system allows for the enhanced delivery of paclitaxel to the tumor, consequently leading to increased bioavailability and alleviated toxicity to normal tissue compared to cremophor-based paclitaxel formulation (Kim 2017). In the combination regimen with GEM, it demonstrated significant improvements in the most clinical outcome parameters (RR: 23% vs 7%; PFS: 5.5 months vs 3.7 months; OS: 8.5 months and 6.7 months) compared to GEM alone. The number of patients with serious adverse events was similar in the two treatment groups (Von Hoff et al., 2013). These results lead to the approval of nab-paclitaxel by FDA as a first-line combination therapy with GEM for patients with locally advanced and metastatic PDAC. Targeting tumor

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stromal cells and modulation of tumor microenvironment by nab-paclitaxel have been proposed as contributing factors underlying the therapeutic activity of nab-paclitaxel in this combination (Figure 1). A clinical study that evaluated the effects of the pretreatment with nab-paclitaxel and GEM combination in patients with operable PDAC showed that improved outcomes of this treatment were in good correlation with reduced tumor stiffness as determined by endoscopic ultrasound elastography (Alvarez et al., 2018). Moreover, in marked contrast with tumors exposed preoperatively to conventional chemoradiation studied for comparison, less abundant collagen matrix and decreased number of tumor-associated fibroblasts around tumor glands were detected in the resected tumor tissues from patients treated with nab-paclitaxel and GEM (Alvarez et al., 2013). Although the number of patients in this study was small, the reported data support the hypothesis that therapeutic benefits of nab-paclitaxel can be associated with stromal distortion. In addition, an active transport mechanisms mediated by albumin cellular receptors may be responsible for the increased intratumoral concentrations of paclitaxel. Other possible mechanisms, like inactivation of GEM catabolizing enzyme, are still under investigation. Since the regulatory approval of nab-paclitaxel /GEM regimen in 2013, this combination has been intensely evaluated in multiple clinical trials in conjunction with a variety of other drugs targeting cancer stem-like cells, tumor microenvironment, kinase and signaling pathways, and immunotherapy (Table 2a and 2b).

Liposomal formulation of irinotecan is another nanomedicine that was approved for use in a combination regimen with 5-FU and folinic acid as a second-line therapy for patients with metastatic PDAC who progressed after gemcitabine-based chemotherapy

(Ko 2016; Kipps et al., 2017; Wang-Gillam et al., 2017). Based on results of a pivotal NAPOLI-1 trial, combination treatment of liposomal irinotecan with 5-FU and folinic acid demonstrated enhanced RR (16% vs. 1%), prolonged PFR (2.3 months vs. 1.4 months, demonstrated as time to treatment failure) and OS (6.1 months vs. 4.2 months) compared to 5-FU/Folinic acid regimen with a manageable safety profile (Wang et al., 2016). No significant OS advantage was observed between patients assigned to liposomal irinotecan monotherapy and those allocated to 5-FU/Folinic acid regimen. Quality-of-life measures did not differ substantially from baseline in any treatment group. An observed excellent performance of liposomal irinotecan led to initiation of clinical studies evaluating the safety, tolerability, and preliminary efficacy of this nanomedicine in combination with other anticancer therapies (Wang-Gillam et al., 2016; Chen et al., 2015; Glassman et al., 2018). For example, the combination of liposomal irinotecan, 5-FU/folinic acid and oxaliplatin, that mimics FOLFORINOX regimen (Rahman et al., 2017), is currently explored in patients with advanced PDAC who have not received prior chemotherapy or as preoperative regimen in resectable pancreatic cancer (NCT03528785, NCT02551991, NCT03487016 and NCT03483038). Another liposomal irinotecan plus 5-FU/Folinic acid based therapies under investigation include combinations with BAX2398 (NCT02697058), Eryaspase (NCT03665441), Cabiralizumab + Nivolumab (NCT03336216), Bermekimab (NCT03207724), Rucaparib (NCT03337087).

Similar to conventional chemotherapy, the treatment regimens involving nanomedicines only modestly improve the overall survival of patients. As our understanding of the complex tumor microenvironment and molecular landscape of PDAC continues to

improve, substantial efforts are undertaken in developing targeted multi-agent therapeutic strategies and drug delivery systems that might improve the effectiveness of PDAC treatment. Novel approaches employing combination therapies discussed in following section, may provide a means of overcoming pathophysiological barriers in patients with PDAC, thus reducing therapeutic resistance.

Targeting desmoplastic stromal barriers

The resistance of human PDAC to systemic therapies is unusual compared with other solid carcinomas and the observed lack of survival benefit might at least partly evolve from the predominant desmoplastic stroma reaction and the pronounced hypovascularity that impede efficient drug delivery to the tumor cells. Thus, normalization of tumor microenvironment represents a promising strategy to improve the penetration and efficacy of systemic chemotherapeutics and/or nanomedicines and may provide important therapeutic outcomes. Such strategies are especially attractive because these pathophysiological barriers cannot be overcome through nanomedicine design alone. One of the approaches to address the stroma is to target the non-cellular components. In this setting, one promising drug is PEGPH20, a recombinant pegylated human hyaluronidase enzyme that is able to degrade hyaluronan, which is an abundant component of the ECM in pancreatic stroma. Preclinical studies in a genetically engineered mouse model of PDAC have demonstrated that enzymatic depletion of hyaluronan by PEGPH20 induced the re-expansion of tumor blood vessels as well as an unexpected selective change in tumor endothelial ultrastructure and macromolecular permeability and resulted in increased the intratumoral delivery of two chemotherapeutic

agents, doxorubicin and GEM. These changes in tumor vasculature and drug delivery also prolonged the survival of the mouse model treated with PEGPH20 and GEM combination compared to GEM monotherapy (Jacobetz et al., 2013). In phase Ib study (NCT01453153) in patients with stage IV untreated pancreatic cancer, PEGPH20 plus GEM combination was well-tolerated and showed promising clinical activity, particularly in patients with tumors expressing high HA levels (Hingorani et al., 2016). Consistent with preclinical studies, the imaging analyses in selected patients demonstrated a rapid increase in tumor perfusion within 24 h after PEGPH20 dosing resulting in higher intratumoral concentration of subsequently administered chemotherapy. The promising results of this trial warranted follow-up studies combining PEGPH20 with other agents. A combination PEGPH20 plus GEM/nab-paclitaxel was evaluated vs. GEM/nab-paclitaxel in an open-label randomized phase 2 trial (HALO 2020) in patents with untreated metastatic PDAC (Hingorani et al., 2018). The study results demonstrated an overall improvement in PFS for patients receiving PEGPH20 plus GEM/nab-paclitaxel. With the limited number of patients, the greatest treatment benefit with PEGPH20 plus GEM/nab-paclitaxel treatment was observed in patients with hyaluronan-high tumors (median PFS of 9.2 month and overall RR of 71%) compared with GEM/nab-paclitaxel control arm (median PFS of 4.3 months; overall RR of 29%). Currently, PEGPH20 is under investigation in the phase III randomized HALO-109-301 trial given in combination with GEM/nab-paclitaxel (NCT02715804) in a biomarker selected patient population with HA-high levels (Figure 1). The limitations of this therapy include thromboembolic events and therefore anticoagulation is now administered in conjunction with PEGPH20 in active clinical trials (Meng et al., 2018; Adiseshaiah et al.,

2016; Hingorani et al., 2015). Collagen is another ECM component that contributes to solid stress and vessel compression in tumors. Jain and his colleagues have demonstrated that the angiotensin inhibitor losartan reduces stromal collagen and hyaluronan production, associated with decreased activity of cancer-associated fibroblasts. Consequently, losartan reduces solid stress in tumors resulting in increased vascular perfusion, increased drug and oxygen delivery and potentiates chemotherapy in PC models (Chauhan et al., 2013). Losartan also increased the efficacy of the liposomal formulation of doxorubicin, Doxil (Diop-Frimpong et al., 2011). Interestingly, the administration of losartan or Doxil alone did not affect the growth of pancreatic tumors, but tumors were significantly smaller in mice treated with losartan plus Doxil combination. These observations are in good agreement with previously reported results of clinical evaluation of liposomal doxorubicin in patients with unresectable PDAC where no complete or partial responses were seen (Halford et al., 2001). Thus, losartan shows potential to enhance the intratumoral penetration and efficacy of small and large therapeutics in patients with PDAC. In parallel, a retrospective clinical data suggested that patients with PDAC receiving GEM monotherapy and treated with angiotensin II receptor blockers or angiotensin-converting enzyme inhibitors to manage hypertension survived approximately 6 months longer than those who were only on GEM therapy (Nakai et al., 2010). Collectively these findings formed a basis of an ongoing phase II clinical trial investigating the combination of losartan and FOLFIRINOX and then chemoradiotherapy in unresectable locally advanced PDAC (NCT01821729) (Figure 1).

Hedgehog signaling has been shown to be active in pancreatic carcinogenesis (Bailey et al, 2008) and its inhibition caused tumor growth suppression that was associated with reduction in the expression of Hedgehog target genes in the stroma compared with control-treated xenograft tumors (Yauch et al., 2008; Feldmann et al., 2008; Mills et al., 2013). Preclinical studies in genetically engineered mouse models have demonstrated that inhibition of Hedgehog pathway signaling transiently increase intratumoral vessel diameter and resulted in stromal disruption and enhanced drug delivery (Olive et al., 2009). Unfortunately, the recent phase II trial (NCT01130142) of GEM plus a sonic Hedgehog inhibitor saridegib versus GEM plus placebo in previously untreated patients with metastatic PDAC has resulted in a disappointing outcome. The trial had to be halted due to progressive disease and decreased median OS in treated with GEM and saridegib (Richards et al., 2012). Furthermore, recently reported data of clinical evaluation of vismodegib, another smoothened inhibitor, and GEM combination failed to demonstrate the benefits over GEM (Catenacci et al., 2015). The reasons for the failure of those drugs in the clinical setting are not entirely clear. Perhaps the stroma is contributing to some tumor control and therefore permanent stroma depletion may also increase the opportunity for cancer cells to metastasize and alleviate stress associated with hypoxia (Özdemir et al., 2014). These results emphasize the need to invest more efforts in the identification of markers of sensitivity upon patient selection as well as development of rational therapeutic combinations and dosing regimens. Overall, pharmacologic inhibition of tumor stroma in combination with chemotherapy is a promising strategy as a means to potentiate delivery of therapeutics to the tumor. However, the ECM is a complex network having both pro- and antitumor effects and a

deeper knowledge of the interactions between stroma and cancer cells is required to translate this strategy into clinical success.

Normalization of tumor vasculature using antiangiogenic agents is another approach for enhancing extravasation of therapeutic agents at tumor sites. The tumor vasculature is abnormal in architecture and morphology of the vascular wall. Tumor vessels are often tortuous and irregular, with poorly aligned defective endothelial cells, detached pericytes, and lack of a smooth muscle layer (Whipple et al., 2008). The leaky nature of tumor vasculature coupled with a dysfunctional lymphatic system forms the physiological basis of the enhanced permeability and retention (EPR) effect, which is beneficial to nanocarriers to extravasate from vessels and accumulate in tumors (Duncan 2006). However, the low tumor perfusion, unevenly distributed blood flow, heterogeneous vessel permeability hinders the homogeneous distribution and deep penetration of the drugs and nanomedicines throughout the tumor (Jain et al., 2012; Miao et al., 2015). Correction of excessive angiogenesis signaling to repair abnormalities in vascular structure and function may enhance drug delivery, thereby increasing chemotherapy activity. The vascular-normalization concept was evaluated in several clinical trials, in which VEGF targeted agents (bevacizumab, axitinib, aflibercept) were added to GEM in treatments of patients with advanced-stage PDAC (Kindler et al., 2010; Sahora et al., 2014; Tian et al., 2013; Rougier et al., 2013). The addition of an agent against VEGFR to GEM-based chemotherapy resulted in higher overall RR but no survival advantage. It is possible that the inhibition of VEGFR was quickly overwhelmed by the signaling forces coming from the other major pathways (EGFR, etc.). It is therefore conceivable that simultaneous targeting of multiple pathways may prevent the rapid emergence of

resistance. As an additional consideration, the chemotherapeutics should be administered during the window of normalization to obtain improved delivery, since vascular normalization is a transient process (Goel et al., 2011).

With respect to the nanocarrier systems, it is well established that the size of the nanomaterial affects the kinetics and extent of tumor accumulation and penetration. Kataoka and colleagues compared the tumor deposition of drug-loaded polymeric micelles of different sizes (30 nm – 100 nm) that exhibited similar circulation profiles and comparable tumor accumulation. It was found that those with diameter above 50 nm mostly accumulated at the periphery of tumors and were not able to efficiently penetrate into the interstitium of poorly permeable, hypovascular tumors such as human pancreatic cancer BxPC3 model consequently resulting in very limited antitumor activity (Cabral et al., 2011; Choi et al., 2007; Choi et al., 2011; Longmire et al., 2008). Conversely, very small nanoparticles with size below 10 nm are often associated with a shorter blood half-life and fast clearance through renal filtration. In general, the vasculature of pancreatic tumor is moderately permeable, with pore sizes in a range of 50–60 nm (Chauhan et al., 2013). Thus, size range of 15-50 nm would be preferable for exploiting the leakiness of the tumor vasculature as far as transport is concerned. Consistent with this concept, cisplatin-incorporated polymeric micelles composed of PEG-poly (glutamic acid) block copolymers with size about 30 nm (under the development name NC-6004, NanoCarrier Co., Ltd.; Japan) were designed to provide sustained release of cisplatin and utilizes the EPR effect to target the release of platinum to tumors (Nishiyama et al., 2003). Preclinical studies have indicated that NC-6004 exhibited prolonged blood circulation, preferential distribution to tumors,

significantly lowered toxicity compared with cisplatin at equivalent doses, and increased antitumor activity (Uchino et al., 2005; Cabral et al., 2011; Baba et al., 2012). These characteristics have been demonstrated in both Phase I study where NC-6004 was used as monotherapy for patients with solid tumors) and a phase Ib/II study where NC-6004 was used in combination with GEM in patients with pancreatic cancer (Plummer et al., 2011; Doi et al., 2017; Subbiah et al., 2018). Based on these results, a Phase III trial is currently ongoing in Asia on the combination therapy of NC-6004 and GEM versus GEM monotherapy in the treatment of patients with locally advanced or metastatic PDAC (NCT02043288) (Figure 1). Another nanomedicine, Genexol®-PM, a 20-50 nm PEG–poly(D,L-lactide) copolymer micellar formulation of paclitaxel, was evaluated as monotherapy in treatment-naive advanced PDAC patients in a single-arm phase II study and demonstrated a median OS (6.5 months) and other efficacy parameters preferable to those seen historically with GEM (Saif et al., 2010). The formulation is currently under evaluation in combination with GEM in patients with recurrent and metastatic PDAC (NCT02739633) (Figure 1).

The contradictory size requirements for prolong blood circulation and improved tumor penetration have aided the development of multistage systems that could maintain relatively large sizes during blood circulation but convert to smaller particles possessing favorable tissue penetration and diffusivity once accumulating at tumor site (Wong et al., 2011; Sunoqrot et al., 2014). For example, Wang and colleagues have fabricated pH-sensitive nanoparticles through amphiphilic polymer directed assembly of platinum prodrug-conjugated polyamidoamine (PAMAM) dendrimers (Li et al., 2016). At physiological pH, these hybrids had size around 100 nm, which favor the long blood

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circulation and enhanced tumor accumulation. Then, acidic tumor microenvironment triggered the release of small PAMAM prodrugs with sizes approximately 5 nm, that enable deep and uniform tumor penetration. Finally, the PAMAM prodrugs are rapidly reduced in the reductive cytosol to release active and potent cisplatin. Proof-of-principle *in vivo* efficacy study conducted in a BxPC-3 human pancreatic tumor model has demonstrated that delivery of cisplatin prodrug via multistage delivery system resulted in superior tumor shrinkage and prolonged survival time compared to free cisplatin or to PAMAM prodrugs loaded in similarly-sized, pH-stable nanoparticles. Moreover, intravital confocal laser scanning microscopy analyses confirmed that the released PAMAM enables efficient extravasation and penetration into deep tumor space (Figure 1). These results emphasize the importance of size on the interstitial diffusivity of particles and suggest that tumor microenvironment-based size switching is a viable strategy for improving drug penetration and therapeutic efficacy especially for the tumors with poor permeability.

Targeting Cancer Stem Cells

Current PDAC treatments fail ultimately, as aforementioned, partially owing to the late diagnosis and dismal prognosis, but also as a result of tumor relapse at local or distant locations after treatments. Recent evidence has demonstrated that a small subpopulation of tumor cells (1-5%) with extremely high tumorigenic potential, pancreatic cancer stem cells (PCSCs), are responsible for the disease progression, its resistance to chemotherapy and radiation, and driving relapse after treatment in PDAC patients (Lonardo et al., 2010; Batlle et al., 2017; Zhan et al., 2015; Hidalgo, 2010).

Moreover, when PCSCs are identified in surgical samples from patients with resectable disease, they confer a shorter survival (Rasheed et al., 2010). Of note, conventional therapies have been found to enrich stem cell fraction in tumors (Batlle et al., 2017). Indeed, several studies have demonstrated that monotherapy with GEM increases the relative proportion of PCSCs (Hermann et al., 2007; Li et al., 2011; Zhang et al., 2016). For example, Hermann and colleagues reported that PCSCs were enriched by > 2 times in the orthotopic L3.6pl pancreatic tumor model following 3-week GEM treatment (Hermann et al., 2007). Thus, strategies aimed at specific targeting and eradication of PCSCs may have important clinical implications. One of the most promising approaches to target and deplete this cell population is certainly the inhibition of stem cell-associated pathways (e.g. Wnt, Hedgehog, mTOR, Notch), and multiple preclinical studies provided compelling rationale for the several Phase I and II trials (Table 2a and 2b). According to the reported data, however, these trials shown very limited positive outcomes with low responses and comparable rates in both PFS and OS. Given these results and our current understanding that all signaling pathways, including those used by PCSCs function as a coordinated network, it is possible that PCSCs are heterogeneous and able to escape inhibition of an individual signaling pathways (Nimmakayala et al., 2018). It is likely that PCSCs targeting will require a design of mechanism-based combination regimens. Funding from the preclinical studies suggest that this hypothesis is worthy of further exploration. For example, Heeschen and colleagues have shown that neither cyclopamine (Hedgehog pathway inhibitor) nor rapamycin (mTOR inhibitor) alone or as supplements to GEM chemotherapy were capable of effectively diminishing the PCSC pool (Mueller et al., 2009). Only combined

inhibition of these two pathways together with GEM, resulted in the desired targeting of the PCSCs. Importantly, administration of this triple combination in mice with established patient-derived pancreatic tumors was reasonably tolerated and translated into significantly prolonged long-term survival. Despite the great efficacy of this combination exhibited in the preclinical setting, the most concerns in the clinic are still the drug related side effects. In this context, utilization of drug delivery systems to overcome some limitations related to drug solubility, stability as well as mitigate off-target toxicity might be beneficial at maximizing drug potentials in eliminating PCSCs. However, the number of reports on nanocarriers as a drug delivery system targeting CSCs in PDAC is rather limited. For instance, HPI-1 is a potent Hedgehog inhibitor based on *in vitro* evaluation. To overcome the limitations related to HPI-1 poor aqueous solubility, its nanoparticle-based formulation (NanoHHI) was developed using PEG-poly(lactic-co-glycolic acid) copolymers. NanoHHI particles of approximately 60 nm in size formed stable aqueous dispersions, and improved systemic bioavailability compared with the parent compound. NanoHHI in combination with GEM suppressed tumor growth in orthotopic Pa03C pancreatic xenografts compared to GEM alone. Importantly, NanoHHI, either as a single agent, or in combination therapy arms, can cause a marked decrease in PCSCs within the tumor suggesting its potential for further translational development (Figure 1). Most recently, Mahato and coworkers designed and evaluated a new analog of vismodegib, MDB5, which was shown to be more potent in depleting PCSCs. In a pancreatic tumor mouse model, treatment with MDB5 containing nanoparticles resulted in significant inhibition of tumor growth and was well tolerated. These results suggest that nanoformulations of MDB5 can be further explored

as a platform for mono and/or combination therapy (Kumar et al., 2017). Drug repurposing for oncology indication has recently been realized due to existing preclinical and clinical data and fast FDA approval (Figure 1). In this context, anthothecol, an antimalarial compound, and its nanoparticle-based formulation were shown to inhibit cell proliferation and colony formation, and induced apoptosis in PCSCs and cancer cell lines, but appeared to have little effect on non-malignant pancreatic ductal cells. It was found that anthothecol inhibits Hedgehog signaling pathway by disrupting binding of Gli to DNA, thus acting as a Gli inhibitor (Figure 1). The other formulations of agents that are effective against PCSCs require further careful evaluations in relevant PDAC animal models to confirm whether their use could potentially improve the treatment outcomes and reduce recurrence of the disease. Other intriguing possibilities include immunotherapy directed against PCSC markers, but a cautious approach is required because many of these markers can be found on normal stem cells in the hematopoietic system (Batlle et al., 2017). Furthermore, regeneration of the PCSC pool by plasticity of non-PCSCs upon treatment cessation presents another challenge for the therapy designs targeting. The development of anti-PCSCs therapies is in a relatively early stage and along with the great promise of drug delivery technologies many issues remain unresolved or unknown and therefore more basic and applied research is needed to translate these therapeutics into clinic (Zhao et al., 2013).

Closing remarks

PDAC remains one of the most devastating cancers with a dismal response to the existing therapies. Despite the intensive research over the past decades, the

development of effective chemotherapeutic treatments has been slow with only modest improvement in patients' survival. Intrinsic and acquired drug resistance extremely shortens the period of effectiveness of drugs whereas those proved to be efficient in other type of cancers (e.g. Taxanes and Anthracyclines) fail in treating PDAC, indicating that PDAC possesses uniquely challenging characteristics that are not yet completely understood. Combination therapies demonstrate improved outcomes in patients' survival and quality of life, nevertheless, the overall improvement is still marginal especially for patients with diagnosed late stages of the disease. With a more comprehensive understanding of physiology and pathology of PDAC, the expansion of treatments beyond conventional chemotherapies to specific and targeted strategies including nanomedicine-based drug delivery platforms hold a great therapeutic promise in combating PDAC. Since proper drug combination can target multiple pathways, promote synergistic drug action, and suppress the development of drug resistance, the use of multiple therapeutic agents in treatment regimen has become the primary strategy to treat PDAC. However, it remains a challenging task due to the possible cumulative toxicities as well as the differences in pharmacokinetics (PK) and biodistribution profiles, which results in inconsistent drug uptake and suboptimal combinatorial effects at the sites of action, that are sensitive to both dosing and scheduling of multiple drugs. Indeed, nanomaterials offer several advantages as therapeutic tool due to design flexibility, small sizes, and can be engineered to interact with specific biological components in targeted diseased tissues. Such carriers can allow simultaneously mixing different drugs in one carrier particle, control drug retention, reconcile the pKs, ratiometric delivery of drug combination and sequential drug release,

which are important determinants for better tailored combinatorial regimens in cancer treatment. This in principle can open very broad possibilities in designing synergistic drug combinations that could affect multiple aberrant pathways in PDAC (Meng et al., 2015; Lu et al., 2017). The selected studies described herein clearly emphasize that nanomedicines can improve the therapeutic responses observed with standard therapies and achieve accelerated clinical translation. However, the complexity of these systems compared to conventional small molecule drugs and intricate structural properties demand careful engineering of the nanocarriers in order to achieve the desired effect. Challenges exist in terms of efficient delivery of the cargo to the tumor as well as clearance of the nanomaterials once they have accomplished their mission *in vivo*. Moving forward, further thorough characterization and understanding of nanomaterial interactions with biological milieus that drive both their intended action and possible toxicological responses and immunogenicity will be critical for the design and optimization of future cancer nanomedicines and will help in eventually taking them from bench to bedside.

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Footnote:

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Figure 1. Targets and therapeutic approaches including conventional and nanotechnology-based drugs that have been approved by FDA (red) or in pre-clinical or clinical evaluation (black) for PDAC treatment.

Table 1. Selected Gemcitabine-based combination therapies evaluated in clinical trials.

Treatment	Target	RR (%)	PFS (months)	OS (months)	Reference
GEM-Based Combination Therapy					
Capecitabine	DNA	19.1 vs 12.4	5.3 vs 3.8	7.1 vs 6.2	Cunningham et al., 2009
		7.3 vs 5.9	-	8.4 vs 7.2	Herrmann et al., 2007
Cisplatin	DNA	26.4 vs 9.2	5.0 vs 2.0	7.5 vs 5.0	Colucci et al., 2002
		11.5 vs 9.0	5.3 vs 3.1	7.5 vs 6.0	Heinemann et al., 2006
		12.9 vs 10.1	3.8 vs 3.9	7.2 vs 8.3	Colucci et al., 2010
Oxaliplatin	DNA	26.8 vs 17.3	5.8 vs 3.7	9.0 vs 7.1	Louvet et al., 2005
		9.0 vs 6.0	2.7 vs 2.6	5.7 vs 4.9	Poplin et al., 2009
Irinotecan	Top I	15.0 vs 10.0	2.8 vs 2.9	6.4 vs 6.5	Stathopoulos et al., 2006
		16.1 vs 4.4	3.5 vs 3.0	6.3 vs 6.6	Rocha Lima et al., 2004
Pemetrexed	Folate metabolism	14.8 vs 7.1	3.9 vs 3.3	6.2 vs 6.3	Oettle et al., 2005
Erlotinib	EGFR	8.6 vs 8.0	3.8 vs 3.6	6.2 vs 5.9	Moore et al., 2007
Cetuximab	EGFR	12.0 vs 14.0	3.4 vs 3.0	6.3 vs 5.9	Philip et al., 2010
Nimotuzumab	EGFR	8.6 vs 8.6	5.1 vs 3.4	8.6 vs 6.0	Schultheis et al., 2017
Bevacizumab	VEGF	13.0 vs 10.0	3.8 vs 2.9	5.8 vs 5.9	Kindler et al., 2010
Ablifercept	VEGF	-	3.7 vs 3.7	6.5 vs 7.8	Rougier et al., 2013
Sunitinib	VEGFR and PDGFR	7.1 vs 6.1	2.9 vs 3.3	7.6 vs 9.2	Bergmann et al., 2015
Axitinib	VEGFR 1-3, c-KIT and PDGFR	5.0 vs 2.0	4.4 vs 4.4	8.5 vs 8.3	Kindler et al., 2011
		4.9 vs 1.6	4.4 vs 4.4	5.1 vs 5.4	Ioka et al., 2015
Sorafenib	VEGFR, PDGFR and Raf	23.0 vs 19.0	5.7 vs 3.8	9.2 vs 8.0	Gonçalves et al., 2012
Vismodegib	SMO	8.0 vs 13.0	4.0 vs 2.5	6.9 vs 6.1	Catenacci et al., 2015
		0	3.7 vs 2.4	6.3 vs 5.4	Catennaci 2012
		21.7	2.8	5.3	Kim et al., 2014
Trametinib	MEK	22.0 vs 18.0	3.7 vs 3.5	8.4 vs 6.7	Infante et al., 2014
Pimasertib	MEK	9.1 vs 9.1	3.7 vs 2.8	7.3 vs 8.3	Van Cutsem et al., 2018
Rigosertib	PLK1 and PI3K	19.0 vs 13.0	3.4 vs 3.4	6.1 vs 6.4	O'Neil et al., 2015
Tipifarnib	Farnesyltransferase	6.0% vs 8.0	3.7 vs 3.6	6.4 vs 6.1	Van Cutsem et al., 2004
Ganitumab	IGF-1R	16.0 vs 10.0	3.7 vs 3.6	7.1 vs 7.2	Fuchs et al., 2015
Evofosfamide	DNA (hypoxia activated)	26.0 vs 12.0	5.6 vs 3.6	9.2 vs 6.9	Borad et al., 2015

		20.0 vs 16.0	5.5 vs 3.7	8.7 vs 7.6	Cutsem et al., 2016
(GEM + Erlotinib) - based Combination Therapy					
Oxaliplatin	DNA	45.0	4.8	8.4	Cascinu et al., 2014
		21.0	5.2	10.5	Yun et al., 2014
Bevacizumab	VEGF	13.5 vs 8.6	4.6 vs 3.6	7.1 vs 6.0	Katopodis et al., 2014
Cixutumumab	IGF-1R	-	3.6 vs 3.6	7.0 vs 6.7	Van Cutsem et al., 2009
(GEM + Cisplatin) - based Combination Therapy					
Sorafenib	VEGFR, PDGFR and Raf	3.4 vs 3.6	4.3 vs 4.5	7.5 vs 8.3	Philip et al., 2014

Abbreviations: RR: Response rate; PFS: Progression free survival; OS: Overall survival; DNA: Deoxyribonucleic acid; Top I: Topoisomerase I; EGFR: Epidermal growth factor receptor; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor; PDGFR: Platelet-derived growth factor receptors; c-KIT: Mast/stem cell growth factor receptor (SCFR); Raf: A family of three serine/threonine-specific protein kinases; SMO: Smoothened, a Class Frizzled (Class F) G protein-coupled receptor; MEK: Mitogen-activated protein kinase; PLK1: Polo-like kinase 1; PI3K: Phosphoinositide 3-kinase; IGF-1R: Insulin-like growth factor 1 receptor.

Table 2a. Nab-PTX/GEM based combination therapy in clinical trials (Completed).

Regimen	Target	RR (%)	PFS (months)	OS (months)	Reference
PCSCs Targeting					
Vantictumab	WNT	53.0	7.2	-	Messersmith et al., 2016
Ipafricept	WNT	29.0	3.9	-	Weekes et al., 2016
Tarextumab	Notch 2/3	19.1 vs 31.8	3.7 vs 5.5	6.4 vs 7.9	O'Reilly et al., 2017
Vismodegib	SMO	43.0	5.5	10	Jesus-Acosta et al., 2014
Sonidegib	SMO	39.0	-	-	Lee et al., 2017
VS-4718	FAK	-	-	-	
Kinase or Signaling Pathways Targeting					
LCL161	Apoptosis Proteins	-	-	-	
Istiratumab	IGF-1R/ErbB3	-	-	-	Ko et al., 2016b
Erlotinib	EGFR	46	5.3	9.3	Leichman et al., 2012
Aptorsen	HSP27 (NF-kB)	18.0 vs 18.0	2.7 vs 3.8	5.3 vs 6.9	Ko et al., 2017
Momelotinib	JAK	28	-	-	Ng et al., 2018
Itacitinib	JAK	24	-	-	Beatty et al., 2018
Target Others					
PEGPH20	HA	45.0 vs 31.0	11.5 vs 8.5	-	Hingorani et al., 2018
Evofosfamide	DNA (hypoxia-activated)	53.0	-	-	Borad et al., 2016

Abbreviations: WNT: Wingless-related integration site; Notch 2/3: Neurogenic locus notch homolog protein 2/3; SMO: Smoothened; FAK: Focal adhesion kinase; IGF-1R: Insulin-like growth factor 1 receptor; ErbB3: Receptor tyrosine-protein kinase, also known as HER3 (human epidermal growth factor receptor 3); EGFR: Epidermal growth factor receptor; HSP27: Heat shock protein 27, also known as heat shock protein beta-1 (HSPB1); NF-κB: nuclear factor kappa B; JAK: Janus kinase; HA: Hyaluronic acid; DNA: Deoxyribonucleic acid.

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Table 2b. Nab-PTX/GEM based combination therapy in clinical trials (Ongoing).

Regimen	Target	Clinic Trial	Phase	Status
PCSCs Targeting				
Sonidegib	SMO	NCT02358161	I/II	U
		NCT01431794	I/II	ANR
Napabucasin	STAT3	NCT02993731	III	R
Kinase or Signaling Pathways Targeting				
Afatinib	ErbB 2 and EGFR	NCT02975141	I	ANR
Ficlatuzumab	HGF	NCT03316599	I	R
FG-3019	CTGF	NCT02210559	I/II	ANR
Ibrutinib	BTK	NCT02562898	I/II	ANR
		NCT02436668	III	ANR
Adavosertib	WEE1	NCT02194829	I/II	ANR
Nintedanib	VEGFR, FGFR and PDGFR	NCT02902484	I/II	R
Ceritinib + Cisplatin	ALK	NCT02227940	I	R
BYL-719	PI3K	NCT02155088	I	ANR
TGR-1202	PI3K-Delta	NCT02574663	I	ANR
9-ING-41	GSK-3β	NCT03678883	I/II	NR
Ulixertinib	ERK	NCT02608229	I	R
Cisplatin + BGB324	AXL	NCT03649321	I/II	NR
Olaratumab	PDGFR-α	NCT03086369	I/II	R
Immune System Targeting				
Tocilizumab	IL-6R	NCT02767557	II	R
Indoximod	IDO	NCT02077881	I/II	ANR
ALT-803	IL-15 superagonist	NCT02559674	I	ANR
Selicrelumab	CD40 antigen stimulants	NCT02588443	I	R
Durvalumab + Tremelimumab	PD-1L + CTLA-4	NCT02879318	II	ANR
Nivolumab + Paricalcitol + Cisplatin	PD-1	NCT02754726	II	R
Oleclumab + Durvalumab	PD-1 + PD-L1	NCT03611556	I/II	R
Cabiralizumab + Nivolumab	CSF1R + PD-L1	NCT03336216	II	R
BMS-813160 + Nivolumab	CCR2/5 + PD-L1	NCT03184870	I/II	R
APX005M + Nivolumab	CD40 agonist + PD-L1	NCT03214250	I/II	R
BMS-813160 + Nivolumab	CCR2/5 + PD-L1	NCT03496662	I/II	R
Pembrolizumab + Paricalcitol	PD-1 + VD receptor activator	NCT02930902	I	R
Other Targets				
Nal-Iri/5-FU + Folinic acid	DNA and Top I	NCT03703063	I	R
Enzalutamide	AR antagonist	NCT02138383	I	ANR
CPI-613	TCA cycle	NCT03435289	I	R
SGT-53	p53 gene	NCT02340117	II	R
ARQ-761	NQO1	NCT02514031	I	R
Selinexor	SINE	NCT02178436	I/II	ANR
Hydroxychloroquine	TLR	NCT01978184	II	ANR
PEGPH20	HA	NCT02487277	II	R
		NCT02715804	III	R
PEGPH20 + Rivaroxaban	HA and Factor Xa	NCT02921022	U	R

Ensituximab	MUC5AC	NCT01834235	I/II	ANR
VCN-01	pRB	NCT02045602	I	R

Abbreviations: U: Unknown; ANR: Active, not recruiting; R: Recruiting; NR: Not recruiting; SMO: Smoothened, is a Class Frizzled (Class F) G protein-coupled receptor; STAT3: Signal transducer and activator of transcription 3, a transcription factor; ErbB2: Receptor tyrosine-protein kinase, also known as HER2 (human epidermal growth factor receptor 2) or HER2/Neu; EGFR: Epidermal growth factor receptor; HGF: Hepatocyte growth factor; CTGF: connective tissue growth factor; BTK: Bruton's tyrosine kinase; WEE1: A nuclear kinase belonging to the Ser/Thr family of protein kinases; VEGFR: Vascular endothelial growth factor receptor; FGFR: Fibroblast growth factor receptors; PDGFR: Platelet-derived growth factor receptors; ALK: Anaplastic lymphoma kinase; PI3K: Phosphoinositide 3-kinase; GSK-3β: Glycogen synthase kinase 3β;ERK: Extracellular signal-regulated kinases; AXL: A cell surface receptor tyrosine kinase, part of the TAM family of kinases; PDGFR-α: Platelet-derived growth factor receptors α; IL-6R: Interleukin 6 receptor; IDO: Indoleamine-pyrrole 2,3-dioxygenase; IL-15: Interleukin-15; CD40: Cluster of differentiation 40; PD-1L: Programmed cell death protein ligand; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; PD-1L: Programmed cell death protein; CSF1R: Colony stimulating factor 1 receptor; CCR2/5: C-C chemokine receptor type 2/5; DNA: Deoxyribonucleic acid; Top I: Topoisomerase I; AR: androgen receptor; TCA cycle: Tricarboxylic acid cycle; P53: Transformation-related protein 53 (TRP53), is any isoform of a protein encoded by homologous genes in various organisms; NQO1: NAD(P)H dehydrogenase [quinone] 1; SINE: Short interspersed nuclear elements; TLR: Toll-like receptors; HA: Hyaluronic acid; Factor Xa: activated form of Stuart-Prower factor; MUC5AC: Mucin 5AC, a protein that in humans is encoded by the MUC5AC gene; pRb: Phosphorylated retinoblastoma protein.

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Figure 1