Pancreatic cancer from bench to bedside: molecular pathways and treatment options

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Abstract: In the last forty years the pancreatic cancer treatment has made advances, however; still novel drugs are needed. It is known that the five year survival rate remains around 5%. The best treatment option still remains surgery, if patients are diagnosed early. In the last decade the biology of pancreatic cancer has been vastly explored and novel agents such as; tyrosine kinase agents, or vaccines have been added as a treatment perspective. The big challenge is now to translate this knowledge in better outcomes for patients. In this current review we will present information from pancreatic cancer diagnosis to molecular pathways and treatment options; current and future.

Keywords: Pancreatic cancer (PC); molecular pathways; targeted treatment

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Introduction

It is known that pancreatic cancer (PC) is a lethal disease with a 5-year survival rate of 1.2–6% encompassing the pancreatic ductal adenocarcinoma that accounts for 85–95% of all pancreatic malignancies (1,2). Current data indicate that it is the seventh and fourth leading cause of cancer-related death worldwide. In the near future by 2030 it will become the second leading cause in the United States (3). During the past 30 years the incidence of pancreatic cancer has increased in developed countries, along with an increase in mortality rates. Increased mortality rates are observed in both sexes (4-7). Current statistical data indicate that there is an increased need for efficient and well-tolerated treatment options in pancreatic cancer. Below we will present current treatment options and the molecular pathways currently investigated. Also, we will provide all novel clinical trials and agents under development that may provide a rationale for future investigations.

Clinical presentation, signs, and symptoms

Unfortunately, pancreatic cancer is usually diagnosed at advanced stage. The most common early disease symptoms are: weight loss, back pain, abdominal pain, nausea and vomiting, dyspepsia, new-onset diabetes, bloating,changes in bowel habit, lethargy,pruritus, shoulder pain, and jaundice (8). In a previous published study, lethargy [OR 1.42 (1.25–1.62)], back pain [odds ratio (OR) 1.33; (95% CI 1.18–1.49)], and new-onset diabetes [OR 2.46 (2.16–2.80)] were observed features of pancreatic cancers (8). The following five symptoms were observed to occur more than 6 months before diagnosis: dysphagia, back pain, changes in bowel habit, shoulder pain, and lethargy (8). In

a previous published study it was observed that lethargy, and depression as the first symptoms in about 38–45% of patients with pancreatic cancer (9). On another occasion a recent review reported nine presenting symptoms in patients with advanced pancreatic cancer (10). In this study abdominal pain and diabetes, were frequently reported in advanced pancreatic cancer (11). Usually in several studies up to 25% of the patients report upper abdominal discomfort up to six months prior to diagnosis (12).

Current management of pancreatic cancer

Surgical resection

Only 20% of patients will be diagnosed with early pancreatic cancer and for these patients, surgical resection is the treatment of choice (13,14). However; after complete resection adjuvant chemotherapy with gemcitabine or 5-fluorouracil and/or chemo-radiation has to follow and still prognosis remains disappointing (15). In the past years randomized controlled trials (16-18) have demonstrated increased overall survival (OS) with adjuvant therapy and this observation is considered the most important advancement in the treatment of pancreatic cancer (13). There are also cases were neoadjuvant chemotherapy is proposed to improve surgical margins in order for the patient to become operable (19-22) (*Table 1*).

Locally advanced and metastatic disease

The chemotherapy agent gemcitabine or gemcitabine-based combination chemotherapy has been vastly used and is the most acceptable first line treatment for advanced pancreatic cancer. However, still the median survival (MS) remains approximately 9 mo (13,23,24). In a recent study "FOLFIRINOX" (folinic acid, 5-fluorouracil, irinotecan, oxaliplatin) an advantage in survival and quality of life was observed when compared to gemcitabine alone. In specific; this combination significantly improved the OS, progression-free survival in pancreatic cancer patients (25). Almost the same effectives was observed with nab-paclitaxel plus gemcitabine (26). It has been observed that almost 10% of patients who have received have survived two years, which is a rare event in advanced disease (27). However, an issue that has to be taken under consideration is the fact that these regimens have increased toxicities and therefore they have to be administered to patients with good performance status (25,26). The care of patients with poor performance status or metastatic disease remains palliative, since gemcitabine based therapies have limited efficacy, however, local therapies can be considered (28).

Targeted therapy

Targeted treatment based on the genome of the tumor has revolutionized current cancer treatment. Pharmacogenetics seems to be one of the treatment options for pancreatic cancer (29). Unfortunately genetically heterogenicity has been observed in pancreatic cancer (30), however; there are several targeted therapies as treatment options, such as; small molecule inhibitors and monoclonal antibodies which inhibit constitutively-active cell surface signaling molecules. Currently results of phase I–III clinical trials presented by Seicean *et al.* (31) did not present favorable results due to the resistance from *KRAS2* mutations and upregulation of alternate signaling pathways (13,32). Until now only the tyrosine kinase inhibitor of epidermal growth factor receptor, "erlotinib", has been approved agent, in combination with gemcitabine, and offers increase in survival of two weeks (33) (*Figure 1*).

Cell metabolism in pancreatic cancer

In order for normal cells to alter their proliferation rate several metabolic pathways are reprogrammed (34). The increased metabolism of cancer cells is one the most important features of this disease that currently is being targeted as treatment (35). Cancer cells adapt their metabolic needs based on their environment. Recent studies have enlighten several metabolic routes and signaling pathways that control tumor progression (36). The most important pathways are considered the following: glutamine regulatory enzymes, Ras signaling, lipid metabolism and autophagy (37-39). Pancreatic cancer as most cancer types are characterized by a high increase in glucose uptake and metabolism (40) and high glycolytic rates the so called "Warburg effect". Moreover; it is known that cancer cells compared to normal cells have specific metabolic dependencies, they have increased need of the amino acid glutamine (41). Kras is responsible for reprogramming glutamine metabolism in PDAC (42). Targeting the Warburg effect has shown that the p53 status of PDAC determined the response to inhibitors of the enzyme lactate dehydrogenase-A (43). Recently it has been investigated and observed that cancer cells not only fuel from glucose and glutamine but also these cells utilize amino acids as well

Table 1 Pancreatic cancer staging (criteria defining tumour resectability status according to NCCN guidelines, version 2.2015)

Resectability status arterial involvement venous involvement
Resectable
No contact with coeliac axis (CA), superior mesenteric artery (SMA), or common hepatic artery (CHA)
No contact with the superior mesenteric vein (SMV), or portal vein (PV) or _180_ contact without vein contour irregularity
Borderline resectable
Pancreatic head/uncinate process
Solid tumour in contact with CHA without extension to CA or hepatic artery bifurcation allowing for safe and complete resection and reconstruction
Solid tumour contact with the SMA of _180_
Presence of variant arterial anatomy (ex: accessory right hepatic artery.) and the presence and degree of tumour contact should be noted if present as it may affect surgical planning
Pancreatic body/tail
Solid tumour contact _180_ with the CA
Solid tumour contact >180_ with the CA without involvement of the aorta and with intact and uninvolved gastroduodenal artery (some members prefer this criteria to be in the unresectable category)
Solid tumour in contact >180_ with the SMV or PV, or in contact _180_ with contour irregularity of the vein or thrombosis of the vein but with suitable vessels proximal and distal to the site of involvement allowing for safe and complete resection and vein reconstruction
Solid tumour contact with the inferior vena cava (IVC)
Unresectable _distant metastasis
Head/uncinate process
Solid tumour contact with SMA >180_
Solid tumour contact with the CA >180_
Solid tumour contact with the first jejunal SMA branch
Body and tail
Solid tumour contact >180_ with the SMA or CA
Solid tumour contact with the CA and aortic involvement
Head/uncinate process
Unreconstructible SMV/PV due to tumor involvement or occlusion (can be due to tumor or bland thrombus)
Contact with most of the proximal draining jejunal branch into the SMV
Body and tail
Unreconstructible SMV/PV due to tumour involvement or occlusion (can be due to tumour burden)

Abbreviations: CA, coeliac axis; SMA, superior mesenteric artery; CHA, common hepatic artery; SMV, superior mesenteric vein; PV, portal vein; IVC, inferior vena cava.

as lipids and protein (44). It has been observed that early breakdown of proteins and subsequent increase in plasma levels of branched-chain amino acids are early events in pancreatic cancer progression as of course observed in other cancer types (45). In a recent published work it was suggested that mitochondrial respiration is another potential functional target to manage pancreatic cancer (46). In the study by Viale *et al*. it was revealed that tumors can relapse 2–4 months after Kras mutation withdrawal. However, it was observed that after Kras mutation extinction, they found a

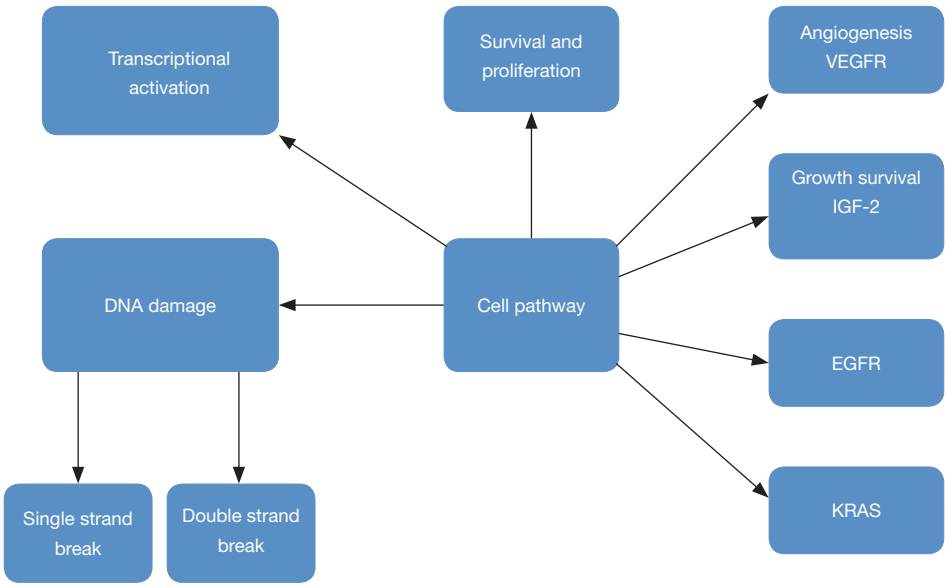

Figure 1 Molecular pathways and treatment. VEGFR, vascular endothelial growth factor receptor; IGF-2, insulin like growth factor-2; EGFR, epidermal growth factor receptor; KRAS, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog, is a GTPase that in humans is encoded by the KRAS.

sub-population of surviving cells that which did not depend on Kras and anabolic glucose metabolism as the primary tumor but they had a strong reliance on mitochondrial energy production (46). Based on this observation it is suggested that in order to eliminate pancreatic tumors and prevent disease relapse we should target both populations, with inhibitors of glucose and mitochondrial metabolism. Mitochondrial metabolism regulation will be the next treatment option for pancreatic cancer. In pancreatic cancer high levels of autophagy are observed due to a transcriptional program that regulates lysosome biogenesis and nutrient scavenging (47,48). Blockage of autophagy in pancreatic cancer was achieved with genetic inhibition and drug administration both *in vitro* and *in vivo* (48,49). However, it has been observed that tumor progression in pancreatic cancer is genotype-dependent and autophagy blockage is not the best treatment option (50). Nevertheless, it is still not established whether p53 status affects PDAC response to autophagy inhibition (51). In pancreatic cancer micropinocytosis of protein and lysophospholipids as source of fatty acids has been observed as a food source. Macropinocytosis is a process where extracellular fluids through specialized vesicles named macropinosomes are inserted into the cells. It has been observed that Ras proteins regulate this process by which cancer cells internalize extracellular proteins, glutamine still remains the most valuable amino acid (52,53). Moreover; Ras-dependent mechanism of scavenging of fatty acid has been shown in pancreatic cancer cells (54). It has been observed that due to the high demand of fatty acids by cancer cells, these cells have found mechanisms of fatty acid supply, such as the uptake of extracellular lipids, in order to face cancer growth requirements. Until now there was an increasing interest in glucose metabolism and aerobic glycolysis, however; it is becoming clearly evident that the alteration of lipid metabolism is critical for cancer cell metabolism (55). Enzymes involved in lipogenesis and lipolysis have been found overexpressed in pancreatic cancer, in particular fatty acid synthase (FASN) that catalyses the final steps of fatty acid synthesis and ATP citrate lyase (39). It has been observed that elevated levels of FASN protein in cancer cells and in serum of pancreatic cancer patients are associated with poor prognosis (39). In cancer cells hyper activation of lipogenesis is observed and characterized by an increase in the degree of lipid saturation compared with non-lipogenic tumors (56). Therefore, the rise in saturated and monounsaturated lipids in cancer cell membranes increases the resistance to oxidative stress, since polyunsaturated lipids are more susceptible to lipid peroxidation. Pancreatic cancer cells use alternative routes to lipogenesis in order to

obtain fatty acids, primarily through uptake of extracellular lipids derived from diet, liver synthesis or released by the adipose tissue. Moreover; current evidences suggest that pancreatic cancer is highly dependent on cholesterol (57). It has been observed that the elevated requirement of cholesterol by pancreatic cancer cells can be supplied by *de novo* synthesis, receptor mediated uptake of cholesterol-rich low-density-lipoproteins (LDL) by the LDL receptor (LDLR), or by hydrolysis of cholesteryl ethers (CE) that accumulate in specific lipid droplets (LDs) that are thought to act as storage of triacylglycerol and CE (57). It has been observed that cholesterol uptake, is activated in tumors (57). Interestingly an increase in the amount of cholesterol and overexpression of the low-density lipoprotein receptor (LDLR) in pancreatic tumor cells is observed (57). Based on this observation LDLR has been proposed as a novel metabolic target to limit pancreatic cancer progression. It has been previously observed in pancreatic tumor cells that shRNA silencing of LDLR reduces considerably cholesterol uptake and alters its distribution, decreases tumor cell proliferation, and limits the activation of ERK1/2 survival pathway. Moreover; with cholesterol uptake blockage the effect of chemotherapy on pancreatic cancer regression to chemotherapeutic agents is increased. It has been observed that high LDLR expression in pancreatic cancer is not associated with tumor stage, however; it has been shown to correlate to a higher risk of disease recurrence. Based on these findings it is suggested that pancreatic cancer cells are highly dependent on cholesterol uptake, and that either this process or LDLR is a promising metabolic target to use in combination with chemotherapy. Lipid metabolism in pancreatic cancer is that pancreatic cancer cells do not rely solely in *de novo* synthesis of lipids but also utilize circulating and diet derived lipids. Therefore it can be said that high dietary intake and obesity is a risk factor in pancreatic cancer.

Pancreatic cancer and its precursors

Pancreatic cancer is usually diagnosed at late disease and therefore it has a poor prognosis with a mortality rate almost equaling to the incidence rate (58,59). Therefore tools for early disease are in need. Moreover; deciphering the factors important for pancreatic cancer progression will help to identify novel treatment options as mentioned in the previous paragraphs. It has been observed that pancreatic cancer can develop from three established precursor lesions (60): (I) intraductal papillary mucinous neoplasm (IPMN); (II) pancreatic intraepithelial neoplasia (PanIN) and the cystic lesions; and (III) mucinous cystic neoplasm (MCN). The majority of pancreatic cancer is thought to arise from PanINs and less frequently from IPMN, whereas MCNs are rare (61). There is indirect evidence for PanINs as precursors for pancreatic cancer, which is largely based on the fact that pancreatic cancer is often associated with advanced PanIN, and both share common tumor promoting genetic alterations. On the other hand, cystic lesions can directly be identified as the origin for PDA on histological examination and imaging techniques such as MRI scan or endoscopic ultrasound (EUS). Due to more frequent and better diagnostic imaging as well as physicians' awareness, IPMN lesions are increasingly identified in the pancreas, the ideal management of these patients is still an ongoing debate (62). It has been observed that pancreatic cancer that is associated with IPMNs has a much more favorable prognosis than pancreatic cancer that arises from PanINs (63-65). A possible explanation could be different genetic mutations during evolution of pancreatic cancer from its precursors. It has been observed that *KRAS* mutation occurs nearly universally during PanIN initiation (66), *KRAS* is less frequently mutated in IPMNs (67,68). IPMNs but not PanINs frequently harbor mutations in *GNAS* and *RNF43* (69-71). Moreover; common genetic alterations in both precursors are found in *TP53* and *CDKN2A* [reviewed by Xiao (72) and Gnoni *et al*. (61)]. Furthermore; the different biology of PanIN and IPMN-associated with pancreatic cancer could be the different cellular origin of the precursors. Recent evidence from genetically engineered mouse models (GEMM) revealed that the cellular origin of PanINs and IPMNs might be different (73-75).

Targeted therapy in PDAC

Epidermal growth factor receptor (EGFR) pathway inhibitors

Epidermal growth factor receptor (EGFR) is a transmembrane receptor member of the ErbB family with a tyrosine kinase domain that is activated by many ligands including epidermal growth factor (EGF), amphiregulin, epiregulin, tumor growth factor-α (TGF-α), heparin-binding EGF, betacellulin and neuregulin. EGFR is involved in cell cycle regulation, adhesion, differentiation and cell survival, through activation of the Ras/MAP kinase, Janus kinase/Stat, phospholipase C/protein kinase C and phosphatidylinositol 3'-kinase (PI3K)/Akt pathways. In previous studies it was observed that EGFR is overexpressed

in up to 90% of pancreatic cancer samples. Based on this findings tyrosine kinase inhibitorstargeting EGFR have been considered a promising therapeutic agent (76). Eroltinib is a tyrosine kinase inhibitor (TKI) molecule that competes with ATP for binding to the kinase domain, thereby blocking downstream signal transduction of EGFR. A large phase III trial, enrolling 569 chemotherapy naïve patients with locally advanced or metastatic pancreatic adenocarcinoma randomized to receive gemcitabine plus placebo or gemcitabine plus erlotinib 100–150 mg daily was performed. The median overall survival (mOS) and progression free survival (PFS) were modestly, but statistically significantly, improved in the combination arm, 6.24 *vs.* 5.91 mo (P=0.038) and 3.75 *vs.* 3.55 mo (P=0.004), respectively (33). Both EGFR status nor KRAS status analysed in the subgroup of patients treated with erlotinib was associated with survival benefit in patients receiving the combination schedule (77).

For now erlotinib has been approved by the FDA in combination with gemcitabine as a first-line treatment for advanced pancreatic adenocarcinoma. Cetuximab is a monoclonal antibody binding the extracellular domain of epidermal growth factor receptor. Unfortunately the encouraging results in a phase I trial, were not observed again in subsequent studies (gemcitabine-based chemotherapy) failed to demonstrate any survival benefit (78,79). Gefitinib; another tyrosine kinase inhibitor of ATP binding to the intracellular kinase domain of EGFR, in combination with gemcitabine in inoperable or metastatic pancreatic cancer patients was administered in a phase II trial. The combination demonstrated promising activity with a mOS and PFS in the combination arm of 7.3 and 4.1 mo, respectively, however; other evidence supporting a role of gefitinib in pancreatic adenocarcinoma treatment is lacking (80). HER-2, which is another ErbB family of transmembrane tyrosine kinase receptors which has been observed overexpressed in 11% of pancreatic adenocarcinoma cases. It has been observed that HER2-positive status is correlated with shorter survival (81). Trastuzumab plus gemcitabine was tested in 34 metastatic pancreatic cancer patients with HER-2 overexpression as determined by immunohistochemistry, and partial responses were observed in 6% of cases (82). Harder *et al.* (83) in a multicentre phase II study, investigated the efficacy and toxicity of the HER2 antibody, trastuzumab, plus capecitabine in patients with pancreatic cancer and HER2 overexpression, however; this study did not present favourable results to either PFS or OS compared with standard chemotherapy. Lapatinib another HER-2 inhibitor was approved by the FDA and clinical trials have been initiated to test the effect of this inhibitor combined with chemotherapy in pancreatic carcinoma. Lapatinib was tested in combination with capecitabine as a second-line treatment in advanced pancreatic cancer with promising initial results. However; more studies are needed to evaluate the real effectiveness and role of this molecule in the treatment of pancreatic adenocarcinoma (84). Nimotuzumab, another anti-EGFR monoclonal antibody, showed promising results (85). In the study by Strumberg *et al.* (86) a phase II trial showed PFS after 1 year of 10.3% and median overall survival of 18.1 wk with a tolerable toxicity profile. Currently afatinib; another TKI of EGFR, HER2 and HER4, is under evaluation in an ongoing phase II trial (87,88).

The KRAS pathway and downstream signalling cascade inhibitors

Current information regarding KRAS mutations indicated that KRAS activating mutations are present in 70% to 90% of pancreatic cancer. K-Ras is a GTPase protein belonging to the Ras protein family. This protein has oncogenic activity, and promotes activation of proliferation and inhibits apoptosis through the RAF/MEK/ERK and PIK3/AKT pathways. It is known that K-Ras pathway is very difficult to target, and currently there are no inhibitors that can actually block this pathway in clinical practice (89). In a preclinical study it was observed that that farnesylation is an important post-translational modification required for Ras activation, allowing the protein to be attached to the plasma membrane for signal transduction (90). Although farnesyl-transferase inhibitors, particularly tipifarnib, presented anti-proliferative activity in pancreatic tumour cell lines, it failed to improve overall survival either as a single agent or in combination with gemcitabine in a phase III trial (91,92). Therefore it is suggested for now to block targets downstream of KRAS, such as the protein kinase MEK. Therefore selumetinib an oral small molecule that inhibits MEK1/2 was produced. This agent was administered in a phase II trial, where patients were randomized to receive single-agent capecitabine or selumetinib as a second-line treatment for advanced pancreatic cancer. The selumetinib arm showed a median overall survival of 5.4 *vs.* 5.0 mo in the capecitabine arm, but this result was not statistically significant (93). Trametinib, another MEK1/2 inhibitor, has been tested in pancreatic cancer in combination with gemcitabine against a regimen of gemcitabine plus placebo in a phase II randomized multicentre study. However; again no significant advantages

were demonstrated in terms of overall survival or PFS (94). Rigosertib, a first-in-class Ras mimetic and small molecule inhibitor of multiple signalling pathways, including polo-like kinase 1 and phosphoinositide 3-kinase (PI3K), was administered in combination with gemcitabine in patients with treatment-naïve metastatic pancreatic adenocarcinoma in a phase II/III randomized study. In the combination regimen no improved of survival or response was observed, as recently presented at the 2015 ASCO Annual Meeting (95). Research in this field is continuing.

IGFR pathway inhibitors

Insulin like growth factor 1 receptor (IGFR-1) is another possible target for pancreatic cancer, which is highly expressed in pancreatic cells. It has been observed that upon ligand binding, it activates several pathways involved in cell proliferation and cell survival such as the PIK3/AKT pathway (96). Moreover, other agents such as; monoclonal antibodies against IGFR (cixutumumab, ganitumab) were evaluated in pancreatic cancer treatment, however; they failed to show a statically significant survival benefit (97). In a phase III trial assessing ganitumab in combination with gemcitabine, the study closed early based on a pre-planned futility analysis. The median overall survival was 7.1 mo in the maximum dose ganitumab arm *vs.* 7.2 mo in the placebo arm (HR 0.97, P=0.397) (98).

Angiogenesis pathway inhibitors

Neo-angiogenesis is known to be an essential metastatization and tumor progression mechanism. Vascular endothelial growth factor (VEGF) is known to stimulate proliferation of endothelial cells and it has been observed to be overexpressed in human pancreatic cancer. However; current data indicate that neo-angiogenesis inhibitors, particularly VEGF inhibitors, have not presented favorable overall survival in combination with gemcitabine in advanced pancreatic cancer. In two phase III trials that tested the efficacy of bevacizumab in association with gemcitabine alone, or gemcitabine plus erlotinib, did not confirm previous findings (99,100). A new recombinant fusion protein, aflibercept which has extracellular portions of VEGFR-1 and VEGFR-2, which binds VEGF-A, VEGF-B and placental growth factors 1 and 2 thereby inhibiting VEGF-ligand-dependent signaling (101) processes, was observed to suppress tumor growth in pancreatic cell lines and xenografts. However; in a phase III study adding aflibercept to gemcitabine did not improve OS in metastatic pancreatic cancer patients (102). Similarly sorafenib, an oral multikinase inhibitor of Raf-kinase, VEGF-R2/-R3 and PDGFR-β, and axitinib, an anti-angiogenesis agent did not present any statistically significant efficacy in advanced pancreatic adenocarcinoma (103,104). Currently there are phase II studies combining chemotherapy with promising new anti-angiogenic molecular agents, such as TL-118, which is a nonsteroidal anti-inflammatory oral medication, or necuparanib, which is re-engineered from heparin with possible anti-tumor activity. The results of these studies will be published soon (105,106).

Embryonic pathway inhibitors

The Hedgehog signaling is known to have a critical role in cell proliferation and survival during the embryonic development. In normal pancreatic cells this pathway is silenced, however; pathological activation is observed in many solid tumors. In pancreatic cancer this signaling pathway is found overexpressed. Hedgehog binds to the extracellular receptor Patched, which, in the absence of Hedgehog, suppresses activation of the G-protein-coupled receptor Smoothened and upregulates glioma associated oncogene homolog1 transcriptional activity (107). In the study by Bailey *et al.* (108) it was observed that Sonic hedgehog (SHH) was detected in precursor lesions and in pancreatic cancer tumor samples which contributed to the formation of the desmoplastic reaction. This characteristic of pancreatic cancer limits the effective delivery of anticancer agents to pancreatic cancer cells. Genetically engineered mouse models demonstrated a depletion of tumor matrix from Sonic hedgehog SHH pathway inhibition this treatment option could be the strategy in pancreatic cancer therapy (109). Currently, vismodegib (GDC-0449), an oral small-molecule inhibitor targeting Smoothened (110), is administered in an open phase II trials in combination with gemcitabine in advanced cancer, in combination with gemcitabine and nab-paclitaxel in metastatic settings. Current results provide favorable outcome (111), and as a single agent in neoadjuvant settings followed by surgery (112-114). The Smoothened inhibitor saridegib (IPI-926) was administered in combination with gemcitabine against gemcitabine plus placebo in a randomized, double-blind, placebo-controlled phase II trial. In this trial patients with metastatic disease were enrolled. The study was terminated due to early decreased of patient survival in the saridegib arm (115). Hedgehog inhibitors are

currently an active research field, and the results of several clinical trials are under way (116). Notch signaling is another embryonic pathway crucial for pancreatic organogenesis, this pathway is silenced after pancreas development, however; it is active only in a stem cell subgroup. This pathway has been observed to be upregulated pancreatic cancer and promotes tumourigenesis. Binding of Notch ligand to its receptor promotes a cascade of proteolytic cleavages, mediated by γ-secretase. The activated form ICN (intra cellular notch) forms part of a transcription complex that, after translocating to the nucleus, regulates transcription of several genes involved in proliferation and differentiation of cells. It has been observed that interacts with other pathways such as Hedgehog, KRAS and NF-κB signaling (117,118). The selective inhibitor RO4929097 of the γ-secretase enzyme has anti-tumor activity in preclinical studies (119). In a recent phase II single-arm trial RO4929097 was assessed, enrolling 18 previously treated advanced pancreatic cancer patients. The treatment was well tolerated; the median survival was 4.1 mo, and the median progression-free survival was 1.5 mo (120). Encouraging clinical results were observed testing demcizumab, an anti-Delta-like ligand 4 antibody, plus gemcitabine and nab-paclitaxel in advanced PDAC in a phase b trial. However; more studies are in need to confirm these preliminary data (121).

Poly ADP-ribose polymerase (PARP) inhibitors

Mutations that affect breast cancer pathway components, and especially the tumor suppressor gene breast cancer-2 (*BRCA2*) gene, which is associated with hereditary predisposition to breast, ovarian and pancreatic cancer are known to promote deficiency in DNA damage repair mechanisms and also induce genome instability (122). Poly ADP-ribose polymerase (PARP) is a nuclear enzyme recruited to repair cell DNA damage, patients with defects in the homologous DNA recombination pathway may benefit from the use of PARP inhibitors. This treatment can be applied as monotherapy or in combination with radiation or other chemotherapeutic agents. Clinical trials testing poly ADP ribose polymerase (PARP) inhibitors are currently under development phase (123-125).

mTOR and PI3K/Akt pathway inhibitors

It is known that after activation, Ras can phosphorylate PI3K, which in turn activates Akt, a serine/threonine kinase. Signal transduction by activated PI3K/Akt plays a role in tumor cell proliferation, metabolism and survival. This occurs through several downstream targets, such as; the mammalian target of rapamycin (mTOR) (126). Currently there are trials testing PI3K/AKT axis inhibitors in advanced pancreatic cancer patients after encouraging preclinical model results (127). There are several PI3K/AKT axis inhibitors being tested such as: RX-0201, an Akt antisense oligonucleotide tested in a phase II study plus gemcitabine; BKM120, a PI3K inhibitor tested in combination with the mFOLFOX-6 schedule; and BEZ235, a combined inhibitor of PI3K and mTOR assessed in a phase study in combination with the MEK inhibitor MEK162 (128-130). In the study by Wolpin *et al.* (131) everolimus, an oral mTOR inhibitor, was administered as monotherapy in 33 gemcitabine-refractory pancreatic cancer patients. The PFS and OS observed were 1.8 and 4.5 mo, respectively. In a recent published phase II study (single arm) where everolimus was tested in combination with capecitabine the median OS was 8.9 mo and PFS was 3.6 mo (132). In the recent future we are anticipating the results of a phase I/II study testing everolimus in combination with gemcitabine in advanced settings and the results of a phase II trial testing temsirolimus (mTOR inhibitor) (133,134).

Tumor stroma inhibitors

In pancreatic cancers a dynamic compartment (stroma) has been observed to be critically involved in tumor formation, progression and metastatic process. Stroma microenvironment and consisting elements are considered a treatment target in addition to previously described trials evaluating Hedgehog signaling inhibitors (135). Currently a phase II trial is recruiting patients I order to administer PEGPH20, a pegylated formulation of recombinant hyaluronidase. The study design is to enrol untreated patients with metastatic disease in order to receive a combination of PEGPH20, nab-naclitaxel and gemcitabine or a combination of nabpaclitaxel and gemcitabine (136,137). Moreover; it has been observed that that inhibition of platelet derived growth factor receptor (PDGFR), could be a possible treatment target. This receptor is expressed in stromal cells and has a critical role in recruiting pericytes and in interstitial fluid pressure in the tumor stroma. The importance of this pathway was suggested by preclinical studies using an orthotopic pancreatic tumor mouse model (138). TKI258, a PDGFR inhibitor, is currently under evaluation in a phase I dose

assessment for advanced pancreatic cancer patients (139). Moreover; matrix metalloproteinases (MMPs) are a family of proteolytic enzymes responsible for the degradation of connective tissue proteins. Matrix metalloproteinase proteins aberrant expression is observed in pancreatic adenocarcinoma. Marimastat has been previously tested. However; the results of a phase III trial which used marimastat with gemcitabine in patients with advanced pancreatic cancer did not present favorable results (140).

Oncolytic virotherapy

It has been suggested that viruses could be a strategic tool for targeting tumors. This is due to the observation that viruses activate similar pathways as tumors, and viral infections activate both the innate and adaptive immune responses (75). It has been observed that tumors create a niche of innate and adaptive immune suppression, which not only protect the tumor from the host immune system. Additionally, it limits its ability to respond to viral infection (141). In early case reports tumor regression was observed following a naturally occurring viral infection (142). The propose strategy suggests that tumor-targeted oncolytic viruses (TOVs) could selectively infect, replicate in, and lyse tumor cells, sparing healthy, normal tissues. There are two approaches for tumor-targeted oncolytic viruses the inherently tumor-selective, *i.e.*, are naturally nonpathogenic to humans and sensitive to antiviral signaling (143) or secondly to depend on oncogenic signaling pathways such as constitutively-activated Ras (144,145). TOVs can also be genetically engineered to be tumor-selective. In order to achieve this deletions of genes are required for replication in normal tissues.(144) Deletion could involve deletion of thymidine kinase *UL23* gene in herpes simplex virus (HSV)-1 (146) or thymidine kinase and vaccinia growth factor (VGF) for vaccinia virus (VV) (147). In recently published literature, gene silencing by RNA interference technology to achieve tumor selectivity has also been utilized (145). Furthermore; another strategy used is to involve the insertion of a tumor-specific promoter (148,149), by doing this the expression of a gene necessary for viral replication in order to restrict its replication in tumor cells is overexpressed (145). TOVs can also be engineered to express cell surface receptors unique to tumor cells (150,151), which allow specific tropism (145). TOVs can designed to express immunomodulatory transgenes (144). In contrast to the pharmacokinetics of the usual drug administration, the therapeutic dose of TOVs increases with time as the virus replicates and spreads to neighboring cells (144). Again as every virus particle carries a therapeutic gene, each viral progeny will also carry the transgene, in this mode it enhances the therapeutic effect (152). TOVs have the ability to directly lyse infected malignant cells and cause acute tumor debulking. However; another major advantage, is the ability of the virus to spread and potentiate an inflammatory response that allows the destruction of a tumor. This feature distinguishes TOVs from vaccines or immune adjuvants (153). Moreover; another unique feature of TOVs is to target silmutaneously multiple cellular pathways and therefore the risk of tumor resistance development is low (144). Another observation suggested that TOVs act synergistically with conventional chemotherapy and radiation (154-156). The first TOV to undergo a clinical trial was ONYX-015 (*dl1520*), an adenovirus deficient in the *E1B* gene (157). The gene product E1B-55 kDa protein was originally thought to sequester p53, inactivating it and as a result, allowing replication in a cell (158). In the study by O'Shea *et al.* (159) later determined that it was for the differential viral RNA export between normal and cancer cells which accounts for ONYX-015's tumor-selectivity. Until now the first approved oncolytic virus to date is H101 (Oncorine; Shanghair Sunway Biotech, Shanghai, China), which was approved for combination with chemotherapy in China in 2005 for the treatment of head and neck cancers (157).

Discussion

The exploration of the genome of pancreatic cancer is currently the best therapeutic strategy as it has important clinical relevance. However; current efforts to understand the tumor genome profile and identify efficient targeted therapies have not presented favorable results. Several targeted agents which have been mentioned in the previous sections, almost all have failed to demonstrate efficacy in late phase clinical trials. Until now only the tyrosine kinase inhibitor erlotinib has been approved by the FDA for advanced pancreatic cancer treatment, however; the improvement of overall survival was barely 2 weeks compared with gemcitabine alone (33). Pancreatic cancer has extreme genomic heterogeneity and this is the most important reason which blocks the identification of new actionable molecular targets or to testing existing biological therapies which have already been approved for human use for other cancers. Since targeted agent administration as a single agent has not presented favorable results,

multitargeted agents multitargeted agents or molecular agent combinations are in the development phase in order to inhibit more than one pathway simultaneously and to prevent or evade resistance. The majority of trials until now have combined target agents with gemcitabine, but actually, the first-line schedules are represented by FOLFIRINOX or gemcitabine plus Nabpaclitaxel. It is suggested that greater efficacy may be obtained from the combination of target agents with those chemotherapeutic drugs. Furthermore, in most studies in which molecular or chemotherapeutic agents in pancreatic cancer were tested, patients from unselected population were enrolled to treat. In the past three years, 116 trials specific for pancreatic cancer systemic therapy were registered of which only about 8% applied criteria to select a patient subset based upon molecular biomarkers (160). In order to stratify patients, the Australian Pancreatic Cancer Genome Initiative has started a pilot study to evaluate the feasibility of assessing a more stratified approach in the management of pancreatic cancer through predefined actionable molecular phenotypes. Patients are enrolled in this trial, called IMPaCT (Individualised Molecular Pancreatic Cancer Therapy). Firstly, a preliminary phenotype screening in performed in order to compare the use of gemcitabine in an unselected population to a stratified approach. The major target of the study is to create a tailored approach to pancreatic cancer treatment, as this seems to be the major challenge for the future of pancreatic cancer treatment (161,162). Moreover; based on the advancements of biotechnology, biological agents can find application in cancer treatment by tumor-targeted delivery of cytotoxic drugs. In the study by Ahn *et al.* (162) a developed antibody fragment was installed in polymeric micelles *via* maleimide-thiol conjugation for selective delivery of platinum drugs to pancreatic tumors. It was observed that this antibody-drug conjugate significantly suppressed the growth of pancreatic tumor xenografts. This technology advancement, which has activity *in vitro* and in a mouse model, could be a promising future strategy in pancreatic cancer therapy not only as a systematic therapy, but also as a local treatment (28,162). In conclusion, the lack of efficacy of targeted therapy in PDAC represents a challenge for the future, and more efforts are needed in order to make pancreatic cancer a curable disease. Future steps include the development of novel conjugates for efficient drug delivery, further exploration of the pancreatic genome and extensive evaluate of combination therapeutic strategies with non-specific cyto-toxic agents and targeted agents in order to maximize clinical benefit (7). The ability of TOVs to stimulate inflammation, deliver genes and immunomodulatory agents as well as reduce tumor burden by direct cell lysis, could be a novel therapeutic approach, however; more trials are in need. Probably this treatment approach could be used simultaneously with non-specific cyto-toxic agents or as an adjuvant treatment. Again local therapies could be considered to reduce tumor burden.

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None.

Footnote

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