The treatment of advanced pancreatic cancer: current evidence and future challenges

M. J. Moore
Princess Margaret Hospital, Toronto, Canada

introduction
Pancreatic adenocarcinoma accounts for 2% of all cancers but 6% of cancer deaths worldwide. In North America it is the fourth leading cause of cancer death with a mortality rate virtually identical to that of its incidence and approximately 36 000 deaths annually [1]. The aetiology is poorly understood, with few validated risk or genetic factors that would assist in defining a high-risk population for screening. Advancing age and cigarette smoking are associated with a greater risk of disease as is a positive family history. Genetic syndromes or germline mutations associated with pancreatic cancer have been recognized (e.g. Peutz–Jeghers syndrome, BRCA2) but are rare and account for a small fraction of cases.

Patients with pancreatic cancer generally present with advanced disease, with less than 20% of patients having potentially resectable disease at diagnosis. Screening and earlier stage detection is currently limited by the lack of biomarkers for pre-malignant lesions and the relative insensitivity of present imaging techniques; although endoscopic ultrasound does show some promise. Historically, surgical resection of pancreatic cancer was associated with high mortality rates; this has improved, and current operative mortality rates are well under 5% in high-volume centres [2]. Underlying comorbidities may still preclude surgical resection in some patients.

Amongst the minority who proceed to potentially curative surgery, the rate of relapse is high, resulting in long-term survival of no more than 10–20% with most eventually dying from uncontrolled systemic disease [3]. This high rate of relapse, coupled with the lack of effective screening strategies and the high numbers of patients presenting with locally advanced or metastatic disease, means that appreciable improvements in outcome in the immediate future can only come if we develop better systemic therapy.

molecular pathogenesis
Pancreatic cancer, even at its earliest stages, is characterized as a very advanced disease from a molecular standpoint, having accumulated a myriad of genetic aberrations in both oncogenes and tumour suppressor genes. Knowledge of these changes defines candidate pathways for novel targeted agents. This is especially relevant in a disease where conventional cytotoxic agents have a limited role. The signature molecular defects initially identified in pancreatic cancer [matrix metalloproteinase (MMP) overexpression, K-ras mutation, epidermal growth factor receptor (EGFR) expression] were the basis of initial trials of targeted agents, and more recently recognized defects such as CDKN2A, TP53 and SMAD4/DPC4, hedgehog signalling and PI3 kinase provide a platform for the further development and investigation of drugs under development that specifically target these pathways [4].

The genetic aberrations characterized to date consist of very complicated chromosomal gains or losses. Interestingly, more chromosome losses have been described in this disease, highlighting the important roles of tumour suppressor genes in the pathophysiology of pancreatic cancer and the possibly smaller role of oncogene activation [5].

Possibly the best characterized mutation in pancreatic cancer is that of the ras oncogene, documented in 75–90% of pancreatic cancers. It is one of the earliest mutations in the pathophysiology of the disease and is often seen in pre-malignant pancreatic lesions [6]. The identification of K-ras mutations in mucinous areas of chronic pancreatitis confirmed a link between this entity and pancreatic cancer that had been previously suggested by epidemiological studies. Ras is a 21-kDa GTP-binding protein, a signalling mediator which, once activated, plays key roles in activating other signalling molecules, thereby being involved in a diversity of cellular functions. There are three mammalian ras genes—H(arvey)-, K(irsten)- and N(euroblastoma)-ras and aberrations in pancreatic cancer take the form of activating mutations in one of these genes, most often in K-ras (least commonly in H-ras). Most involve a specific mutation in codon 12 (from GGT to GAT or GTT or more rarely, CTT)—it is this mutation which results in a substitution of glycine with aspartate, valine or arginine, that is considered the so-called 'signature' of pancreatic cancer.

Once mutated, Ras becomes constitutively activated, promoting the hallmark oncological processes of cell proliferation, survival and differentiation through activation of the mitogen-activated protein (MAP) kinase cascade. The ability of Ras to mediate downstream signalling is by virtue of its recruitment and activation of Raf, which in turn signals via MAP-K (MEK/ERK).

The hedgehog family of genes, which includes sonic hedgehog (Shh), encodes signalling molecules that have key roles in development and organogenesis. New evidence suggests...
that activation of sonic hedgehog signalling is a critical early
mediator of pancreatic cancer development. Binding of
the ligand to its receptor, leads to activation of transcription factors
that participate in the regulation of gene expression [7]. Gains
in chromosome 20q have been noted in pancreatic cancer,
implicating the possible existence of an oncogene at that locus.
A potential candidate for this position is AURKA, which
encodes a kinase (Aurora-A) with key roles regulating mitotic
proteins including kinetochores, centrosomes and spindle fibre.
In vitro studies demonstrate that amplification of AURKA
 correlates with chromosomal instability in pancreatic cancer
cell lines [8]. In addition, further cell line studies have shown
an antitumour effect and potentiation of taxane
chemosensitivity with knockdown of AURKA levels by RNA
interference [9].

Aberrations of 9p21, which harbours the gene for CDKN2A/
INK4A/p16 are often observed in pancreatic cancer. These
aberrations are mostly ‘inactivation’ by deletion or mutation,
but in some cases gene ‘silencing’ by hypermethylation.
CDKN2A encodes a cyclin-dependent kinase inhibitor, which
induces cell cycle arrest at G1 in cooperation with normal Rb
(retinoblastoma) function [10]. Animal model work suggests
that loss of CDKN2A is an early event in pancreatic cancer
(being noted in less well differentiated benign pancreatic
pathology) which functions to enhance the oncogenic effect of

The SMAD4 gene encodes a transcription factor that plays
central roles in transforming growth factor beta (TGFβ),
bone morphogenetic protein (BMP) and activin signalling
pathways [12], and its inactivation is noted in about 50% of
pancreatic cancers. The biological role of SMAD4 mutations is
currently under active investigation, with opposing views
regarding its clinical and pathological implications having
being published in the past. More recent reports suggest that
wild-type SMAD4 functions to constrain activated K-ras-
initiated neoplasms which rapidly progress to frank invasive
disease in the context of SMAD4 deficiency [13].

Loss of activity of tumour suppressor p53/TP53, with key
roles in cell cycle arrest and apoptosis, has been reported in
numerous tumour types including pancreatic. Missense
mutations in the DNA-binding domain of this transcription
factor result in the production of a protein that is able to
translocate to the nucleus but unable to function in DNA
binding and therefore accumulates in the nucleus. This
accumulation has been described in high-grade and late intra-
epithelial lesions, implicating this mutation at later stages of
pancreatic carcinogenesis [14]. As with SMAD4 and p16
deletions, loss of p53 induces further chromosomal instability
in the context of mutated, activated K-ras.

**systemic therapy**

Gemcitabine has been the standard systemic therapy for
unresectable pancreatic cancer since the late 1990s. In the
landmark study reported by Burris and colleagues 126
treatment-naïve patients with unresectable pancreatic cancer
were randomized to receive either intravenous gemcitabine or
5-fluorouracil (5-FU). The primary outcome, clinical benefit,
a composite outcome consisting of measurements of pain,
weight and performance status, was significantly better in
gemcitabine-treated patients (24% vs 5% in patients receiving
5-FU). In addition there was a small but statistically
significant improvement in median survival (5.65 months vs
4.41 months) with a 1-year survival of 18% in gemcitabine-
treated patients in comparison with 2% in patients receiving
5-FU (P < 0.002) [15].

The combination of gemcitabine with other
chemotherapeutic drugs such as the platins (cisplatin or
oxaliplatin) [16], additional antimetabolites (pemtrexed,
5-FU) [17] and topoisomerase inhibitors (irinotecan) [18] has
not lead to any significant impact on survival in published
randomized trials. Subset analyses done post-hoc have
suggested some benefit from combinations of gemcitabine
with cisplatin or capcitabine in patients with performance
status 0, although this is the minority of patients. The poor
responses to conventional therapeutics highlight the
importance of alternative strategies in treating this disease
and make the potential of using novel targeted therapies
appealing in this disease site.

**targeted therapies that have been
evaluated in pancreatic cancer**

The identification of drugs targeting ras remains a challenge.
Ras proteins need to undergo the process of farnesylation,
which allow them to then become prenylated to attach to the
plasma membrane, a step that is required for activation. The
farnesyl transferase inhibitors (such as R115777 or tipifarnib)
were designed to impair farnesylation, thereby abrogating ras
signalling [19]. A Phase II study of tipifarnib as a single
agent did not show appreciable activity [19]. The drug was
then investigated further in a randomized, double-blinded
placebo-controlled study comparing gemcitabine ± tipifarnib
in 688 treatment-naïve patients. This study demonstrated no
statistically significant difference in overall survival, objective
disease response or progression-free survival [20]. It has
been hypothesized that tipifarnib was not effective because
other pathways exist to activate the ras proteins beyond
farnesylation or that as K-ras mutation is an early event in the
pathogenesis of pancreatic cancer it is possible that as the
cancer progresses it becomes less dependent on this pathway.

One of the hallmarks of pancreatic cancer is extensive local
invasion, even at early stages of disease, mediated in part by
MMPs, a large family of zinc-dependent proteolytic enzymes.
**In vitro** work showed a reduction in invasive ability without
a corresponding affect on proliferation of MMP inhibition on
pancreatic cell lines, and early MMP inhibition in orthotopic
mouse models resulted in fewer metastases and a survival
advantage to these animals [21]. These preliminary studies led
to early clinical evaluation of this class of drugs. These were
universally disappointing. In one study 414 advanced
pancreatic cancer patients were randomized to one of three
doses of the MMP inhibitor marimastat or standard dose
gemcitabine [22]. There was no improvement in median
survival and the trend was to inferior outcomes with
marimastat when compared with gemcitabine. In a second
study of 239 patients with unresectable pancreatic cancer
there were no improvements in overall survival or response
rates with the addition of marimastat to gemcitabine [23]. BAY 12-9566 is a specific inhibitor of MMP-2, MMP-3, MMP-9 and MMP-13 that also has anti-angiogenic properties. A National Cancer Institute of Canada (NCIC) study compared gemcitabine with BAY12-9566 in patients with advanced pancreatic cancer. At a pre-planned interim analysis when 277 patients had been enrolled it was observed that patients on the MMP inhibitor had a significant survival disadvantage with a median survival of 3.2 months versus the 6.4-month survival of patients on gemcitabine. These results led to early termination of the study [24].

The EGFR family consists of four distinct members, one of which is EGFR, also referred to as ErbB1 or HER-1. EGFR is a 170-kDa transmembrane glycoprotein with an amino-terminal extracellular ligand-binding domain linked to a carboxy-terminal catalytic cytoplasmic domain. Upon ligand binding, the activated receptor then recruits and activates through phosphorylation a variety of intracellular adaptor molecules and signalling mediators and in this way can participate in a literal web of molecular interactions including cell survival, proliferation, survival and motility. The two major downstream pathways instrumental in EGFR signalling are the Ras–Raf–MAPK and the PI3K/Akt pathways. The EGF receptor (HER1/EGFR) is overexpressed in many pancreatic tumours and has been found to correlate with poor prognosis and disease progression [25]. This biological rationale is supported by pre-clinical studies in which antagonizing EGFR signalling was found to inhibit growth and metastasis of pancreatic tumours in xenograft animal models [26].

Two therapeutic approaches have been taken to antagonize signalling through EGFR; the first has involved antagonizing ligand binding using monoclonal antibodies, the second using small-molecule tyrosine kinase inhibitors that compete with ATP for binding to the kinase domain, thereby preventing activation of the receptor and downstream signalling. Erlotinib (Tarceva) is a small-molecule EGFR tyrosine kinase inhibitor that underwent Phase I testing with diarrhoea and skin rash as dose-limiting toxicities. Phase I combination studies with gemcitabine did demonstrate a disease activity in pancreatic cancer and the combination was taken through to Phase III testing [27, 28]. NCIC.PA3 was an international, multicentre double-blind randomized control trial in which 569 treatment-naïve patients with metastatic pancreatic cancer were randomized to receive standard dose gemcitabine plus erlotinib (at a daily oral dose of 100 mg) or placebo. There was a small but statistically significant increase in overall survival from missing data (hazard ratio 0.82; P = 0.02), the primary endpoint of the study. Improvements in secondary endpoints of progression-free survival and disease control rate were also seen. The treatment was well tolerated but there was a higher frequency of adverse events, most of which were mild to moderate consisting of grade 1 or 2 rash, diarrhoea, infection and stomatitis. EGFR status was not associated with drug response or disease stability. Studies exploring other molecular predictive markers are ongoing. Patients who developed a rash on erlotinib had a higher likelihood of achieving disease control, with a median survival of 10.5 months in those with 2+ rash versus 5.3 months in those without rash. Although the improvements in outcomes in NCIC.PA3 were not large, this study validated EGFR as a target in pancreatic cancer has provided further rationale for the on-going study of other molecular agents [29].

Cetuximab (C225) is a chimeric monoclonal antibody that through binding to the extracellular domain of EGFR also blocks signalling through this pathway. It is currently approved for use in colorectal and head and neck squamous cell carcinoma. Pre-clinical data show that cetuximab can enhance the antitumour effect of gemcitabine in xenograft animal models of pancreatic cancer [30]. A subsequent Phase II trial enrolled 61 treatment-naïve patients with locally advanced and metastatic pancreatic cancer who had immunohistochemical evidence of EGFR expression. Patients received weekly gemcitabine in combination with cetuximab (initial dose 400 mg/m², then 250 mg/m² weekly). Seventy-six per cent of patients had disease control with 12% being partial responses and 63% stable disease. The median survival of this cohort was 7.1 months [31]. At GI ASCO 2007 preliminary results of a Phase II trial of cetuximab with gemcitabine/oxaliplatin were reported. In 64 evaluable patients, there was an overall disease control response rate of 38% (with 19% being partial responses) and a 54% 6-month survival [53].

The Southwest Oncology Group (SWOG) recently completed a randomized Phase III trial in 704 patients to answer the question of whether or not the addition of cetuximab to standard dosing gemcitabine would lead to improvements in overall survival. Unfortunately, this study did not meet its primary endpoint of a survival benefit nor were there improvements in secondary endpoints [32].

Agents have been developed targeting other members of the EGFR family, particularly ErbB2 or Her2/neu. The best-known agent is that of trastuzumab, a monoclonal antibody to ErbB2, that is approved for use in both metastatic and adjuvant breast cancer. Evidence to date suggests that trastuzumab is most effective in Her2/neu over-expressing cells and since over-expression of this receptor has been reported in around 20% of pancreatic cancers (in comparison with normal tissue) there is rationale for conducting studies of this drug in this disease site. In a study of gemcitabine + trastuzumab in pancreatic cancers over-expressing Her2/neu, responses were observed in 2 of 32 patients, the median survival was 7 months, and the 1-year survival was 19%, similar to what would be expected with gemcitabine alone. Only 16% of patients evaluated had HER-2/neu overexpression [33].

The concept of targeting angiogenesis as cancer therapy was first suggested by Folkman in the 1970s [34]. He proposed that solid tumours would remain dormant in the absence of neovascularization, and that targeting tumour-derived ‘angiogenesis factors’ would be of therapeutic benefit. One of the central players in the process of angiogenesis is vascular endothelial growth factor (VEGF), also known as vascular permeability factor. VEGF is a 45-kDa secreted glycoprotein, with VEGF-A being of prime importance in angiogenesis. The VEGF pathway can be targeted in a variety of ways, with the most widely used being monoclonal antibodies (bevacizumab) to the ligand or small-molecule VEGF-A...
tyrosine kinase inhibitors (sorafenib). Bevacizumab is a recombinant monoclonal antibody directed against the VEGF ligand. Pre-clinical studies demonstrated that bevacizumab was effective at suppressing growth of pancreatic tumours. An initial Phase II study of bevacizumab with gemcitabine in 52 patients with previously untreated metastatic pancreatic cancer revealed a 21% partial response rate and 46% stable disease with a median survival of 8.8 months [36]. This study provided rationale for the subsequent Phase III trial, CALGB 80303, initially presented at GI ASCO 2007 [37]. Despite the encouraging results in Phase II testing, this double-blinded, randomized Phase III study of 602 patients failed to show a survival benefit with the addition of bevacizumab to gemcitabine (5.7 months in bevacizumab/gemcitabine versus 6.0 months with gemcitabine monotherapy). One possible explanation for the negative result is that the initial Phase II patients had an overall better performance status than the typical pancreatic population and that this rather than the intervention may have accounted for the better outcome.

The other approach to targeting the VEGF pathway involves the use of tyrosine kinase inhibitors that operate on an intracellular level. Two such agents have already been approved for use in other disease sites—sorafenib in advanced renal cell carcinoma and sunitinib in renal cell and gastrointestinal stromal tumours. Sorafenib is a multitargeted tyrosine kinase inhibitor with inhibitory effects against Raf-1 kinase, VEGFR-2 and PDGFR. With its multiple targets it is proposed to exert its antitumour effect via both antiproliferative and anti-angiogenic mechanisms. A recent study evaluated the combination of gemcitabine with sorafenib in patients with advanced solid tumours. This study included an expanded cohort of pancreatic cancer patients. Unlike the normal Phase I study population, the 23 patients in the pancreas cohort were, for the most part, naive to systemic therapy. In this expanded cohort, 13 patients (56.5%) achieved disease stability [37]. At GI ASCO 2007 preliminary results of a first-line efficacy Phase II trial were reported. Disappointingly, although there was a 23% rate of disease stability there were no objective responses to this combination, and median survival was 4 months with a 23% 6-month survival [38].

agents in early phases of clinical testing

Numerous other agents targeting a variety of pathways are currently in pre-clinical and clinical development for antitumour efficacy in pancreatic cancer. Src was the first reported proto-oncogene. It encodes a multifunctional non-receptor tyrosine kinase with key involvement in the regulation of physiological and oncogenic processes including proliferation, differentiation, survival, motility, angiogenesis, local invasion and cell–cell and cell–matrix interactions. Malignant aberrant src activity is related to over-expression of the wild-type protein, as opposed to expression of a mutated genotype. A more malignant phenotype is promoted through the interaction of src with other signalling mediators [39]. Src inhibitors are now in Phase I and II testing in the clinic and NCI Canada is testing an orally available src inhibitor in Phase I/II testing in combination with gemcitabine in patients with advanced pancreatic cancer.

Another substrate of current interest is focal adhesion kinase (FAK). FAK is a non-receptor tyrosine kinase (NRTK) that co-localizes to sites of integrin clustering at focal adhesion junctions connecting cells to the extracellular matrix (ECM). Integrins lack catalytic ability; however, the kinase activity of FAK allows for intracellular signaling in response to stimuli from the extracellular matrix. FAK has been implicated in a broad range of cellular functions from cell survival and proliferation to invasion and migration, in both normal and tumour cell contexts [40]. FAK inhibitors are currently in Phase I testing. Agents targeting other pathways that have been identified as important in the molecular pathogenesis of pancreatic cancer such as MUC4, sonic hedgehog, mesothelin, aurora kinase, MEK and ERK are similarly in the early stages of clinical investigation.

summary

The outcome of pancreatic cancer continues to be disappointing with poor long-term survival even in patients with resectable disease at presentation. The high relapse rate despite the aggressive standard of surgery means that the majority of patients will require, and receive, some form of systemic therapy over the course of their illness. The standard of care for the past decade has been single-agent gemcitabine; a regimen approved based on a demonstration of both clinical benefit and improved survival. Since then, despite testing of numerous other targeted and cytotoxic agents only one—the combination of erlotinib, a small-molecule tyrosine kinase inhibitor directed at the epidermal growth factor receptor—has shown a significant improvement in overall survival.

Given the extensive genetic aberrations present in pancreatic cancer it is reasonable to think that targeting one pathway will be of limited utility due to redundancy of signalling. Rationale drug combinations or the testing of multitargeted molecular agents should result in much greater clinical efficacy by simultaneously inhibiting several of the multiple pathways driving oncogenesis and is the appropriate way to move forward against this deadly disease. Agents targeting most of the pathways critical in pancreatic cancer are now in the clinic and offer hope for a better outcome in the future.

disclosures

The author is conducting research sponsored by Roche/OSI and has consulted for them.

references

symposium article


