Targeted therapies for pancreatic cancer

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Introduction: Pancreatic cancer is a devastating malignancy and a leading cause of cancer mortality. Furthermore, early diagnosis represents a serious hurdle for clinicians, as symptoms are non-specific and usually manifest in advanced, treatment-resistant stages of the disease.

Sources of data: Here, we review the rationale and progress of targeted therapies currently under investigation.

Areas of agreement: At present, chemoradiation regimes are administered palliatively, and produce only marginal survival benefits, underscoring a desperate need for more effective treatment modalities.

Areas of controversy: Questions have been raised as to whether erlotinib, the only targeted therapy to attain a statistically significant increase in median survival, is cost-effective.

Growing points: The last decade of research has provided us with a wealth of information regarding the molecular nature of pancreatic cancer, leading to the identification of signalling pathways and their respective components which are critical for the maintenance of the malignant phenotype.

Areas timely for developing research: These proteins thus represent ideal targets for novel molecular therapies which embody an urgently needed novel treatment strategy.

Keywords: pancreatic cancer/targeted therapy/novel therapy/clinical trials

Introduction

With mortality rates almost identical to incidence rates, a diagnosis of pancreatic cancer represents a particularly bleak prognosis for patients and their families. The factors that contribute towards this poor outcome include our current inability to diagnose pancreatic cancer at early stages due to the non-specific presenting symptoms, the inaccessibility of the pancreas and the early occurrence of metastasis. At present, surgical resection represents the only curative option for patients with pancreatic cancer, but at present, only 20% of patients present with early-stage operable tumours.1 Patients with unresectable disease consequently make up the overwhelming majority of pancreatic cancer...
diagnoses and receive palliative chemotherapy and radiotherapy, which
do not result in significant long-term survival. The paucity of curative
therapies has translated into an overall 5-year survival rate of less than
5%, underscoring a desperate need for improved therapeutic options
and diagnostic tools. Recent work has led to significant advances in the
understanding of the genetic changes characteristic to pancreatic cancer
in the hope of identifying molecules for the development of targeted
therapies, although progress towards translating this knowledge into
effective diagnostic tools and therapeutic agents has been slow. Here, we
review the rationale for new therapies currently being developed.

**Diagnosis and current treatment**

At present, the anti-metabolite gemcitabine (2',2'-difluorodeoxycytidine)
is the standard treatment for patients with pancreatic cancer.
Gemcitabine is a fluorinated analogue of deoxycytidine which, when
incorporated into DNA, inhibits DNA synthesis and repair, culminating
in apoptosis. A landmark study of 126 patients in 1997 demonstrated
that gemcitabine produces significant benefits over its predecessor
5-fluorouracil (5-FU) in patients with advanced pancreatic cancer with
respect to overall survival and disease-related symptoms. Overall
however, gemcitabine produces only marginal survival benefits and
modest improvements in quality of life, representing a necessity for the
identification of combination partners in order to improve the clinical
outcome. To this end, several clinical trials using gemcitabine in combi-
nation with 5-FU, cisplatin, irinotecan, oxaliplatin and pemetrexed
have been conducted, but none of these phase III trials have reported
statistically significant improvements in survival over gemcitabine mono-
therapy. Thus, novel therapeutic strategies are urgently needed. As
locally advanced and metastatic pancreatic cancers are currently incur-
able with standard therapies, experimental therapy is an acceptable
first-line strategy. To this end, researchers are currently focusing their
attentions on targeting the molecular defects characteristic to the malig-
nancy itself, which represents a particularly promising avenue for future
research (Tables 1 and 2).

**Introduction to targeted therapies**

The recent success of novel cancer therapeutics based on the specific
inhibition of tumour-associated pathways has breathed new life into
the area of cancer drug discovery. As these agents have been designed
to inhibit specific pathways, they are commonly referred to as targeted
agents. Conventional therapies also target cellular processes, but fail to distinguish tumour cells from their normal counterparts. This lack of discrimination subsequently manifests in unavoidable, often unacceptable side-effects which can be as devastating to the patient as the disease itself. In contrast, targeted therapies disable specific cellular pathways that are absolutely required for the maintenance of cancer. As a consequence, normal cells are left relatively unharmed, leading to a different and generally less severe repertoire of drug-related toxicities. A further requirement of targeted therapies is that their effects should be quantifiable, with a correlation between target inhibition and clinical outcome, although this does not always occur due to the complexity of the living organisms.

**Telomerase**

Unlimited proliferation is an acquired trait intrinsic to the survival of cancer cells, and mammalian cells have evolved sophisticated, cell-autonomous mechanisms to limit their replicative potential. In *vitro*, human cells undergo a certain number of population doublings (PD) before entering an irreversible post-mitotic state known as replicative senescence. Senescence represents the first of two checkpoints that serve as barriers against limitless proliferation and is dependent on the p53 and retinoblastoma tumour suppressor pathways and can thus be abrogated through either genetic mutation or the expression of viral oncoproteins such as the SV40 T-antigen. Such events allow cells to proliferate for a further 20–40 PD, although the
Table 2 Phase III pancreatic cancer clinical trials with targeted and standard therapies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient number</th>
<th>Disease stage</th>
<th>Treatment</th>
<th>Mechanism</th>
<th>MS (months)</th>
<th>PFS (months)</th>
<th>1-year survival (%)</th>
<th>CR + PR (%)</th>
<th>Stable disease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALGB 80303 (2007)</td>
<td>602</td>
<td>Advanced</td>
<td>Bevacizumab + gemcitabine</td>
<td>Anti-VEGF antibody</td>
<td>5.7</td>
<td>4.8</td>
<td>13.1</td>
<td>40.7</td>
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<tr>
<td>Van Custem et al. (2004)</td>
<td>688</td>
<td>Advanced</td>
<td>Tipifarnib + gemcitabine</td>
<td>RAS farnesyltransferase inhibitor</td>
<td>6.0</td>
<td>4.3</td>
<td>11.3</td>
<td>35.7</td>
<td></td>
</tr>
<tr>
<td>SWOG S0205 (2007)</td>
<td>766</td>
<td>Advanced</td>
<td>Cetuximab + gemcitabine</td>
<td>Anti-EGFR antibody</td>
<td>6.0</td>
<td>3.6</td>
<td>24.0</td>
<td>8.0</td>
<td>52.0</td>
</tr>
<tr>
<td>NCIC CTG (2007)</td>
<td>569</td>
<td>Advanced</td>
<td>Erlotinib + gemcitabine</td>
<td>EGFR tyrosine kinase inhibitor</td>
<td>6.24*</td>
<td>3.75*</td>
<td>23.0*</td>
<td>8.6</td>
<td>48.9</td>
</tr>
<tr>
<td>Chau et al. (2006)</td>
<td>A–18</td>
<td>Advanced</td>
<td>Gastrazole</td>
<td>Gastrin receptor antagonist</td>
<td>7.9*</td>
<td>3.55</td>
<td>17.0</td>
<td>8.0</td>
<td>41.2</td>
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<td>NCIC CTG (2003)</td>
<td>277</td>
<td>Advanced</td>
<td>Gemcitabine</td>
<td>MMP inhibitor</td>
<td>6.7</td>
<td>3.9</td>
<td>13.0</td>
<td>28.7</td>
<td></td>
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<tr>
<td>Bramhall et al. (2001)</td>
<td>414</td>
<td>Unresectable</td>
<td>Gemcitabine</td>
<td>MMP inhibitor</td>
<td>6.59*</td>
<td>3.5*</td>
<td>25.0</td>
<td>5.2</td>
<td>53.9</td>
</tr>
<tr>
<td>Bramhall et al. (2002)</td>
<td>239</td>
<td>Unresectable</td>
<td>Gemcitabine + erlotinib + gemcitabine</td>
<td>Anti-VEGF antibody and EGFR tyrosine kinase inhibitor</td>
<td>5.4</td>
<td>3.1</td>
<td>17.0</td>
<td>16.0</td>
<td>56.0</td>
</tr>
</tbody>
</table>

MS, median survival; PFS, progression-free survival; PR + CR, partial and complete response; CALGB, Cancer and Leukaemia Group B; SWOG, Southwest Oncology Group; NCIC CTG, National Cancer Institute of Canada Clinical Trials Group.

*Statistically significant.
population eventually enters replicative crisis, the second checkpoint, characterized by chromosome instability and a decline of the cell population as apoptosis exceeds proliferation. The rampant genomic instability observed during crisis can be attributed to the attrition of telomeres, which are specialized, tandemly repeated sequences (TTAGGG in humans), located at the ends of chromosomes. The inability of DNA polymerase to replicate the extreme 5’ termini of chromosomes results in progressive telomere shortening with each replicative cycle. Once telomeres become critically short, the cell loses the ability to protect the ends of its chromosomes and enters crisis. Thus, telomere erosion poses a finite lifespan on the cell and represents a serious hurdle for any cell with malignant aspirations. Tellingly however, almost all immortal cells express telomerase, a unique ribonucleoprotein which catalyses the addition of de novo telomeric sequences to the ends of chromosomes and are consequently able to bypass crisis. In contrast to healthy somatic cells, most malignant cells show detectable telomerase activity. Furthermore, the inhibition of telomerase in tumour cells leads to rapid telomere shortening and apoptosis. Taken together, these data provide a rationale for the development of antitelomerase therapies for the treatment of cancer.

Several groups have demonstrated the up-regulation of telomerase in pancreatic malignancies but not in normal tissues or in benign disease. These in vitro studies thus argued in favour of the development of telomerase inhibition for pancreatic cancer treatment. One strategy currently in development is the use of immune recognition technology to target and destroy telomerase-positive cells. hTERT, the telomerase reverse transcriptase catalytic subunit, is degraded into short peptides by tumour cells of different histological origins and types and is presented on the cell surface on major histocompatibility complex (MHC) molecules, leading to the activation of cytotoxic T-lymphocytes (CTLs), resulting in the elimination of tumour cells bearing the same peptide-MHC complex on their surfaces. A significant concern regarding telomerase-based therapies is the possibility of adverse effects of telomere inhibition on the small number of tissue types that do express telomerase such as germline cells, haematopoietic cells and those in stem cell compartments of the skin and intestine. Importantly, no CTL effect was observed towards CD34-positive haematopoietic cells, suggesting that the high expression of telomerase in malignant tissue compared with normal tissue does indeed provide the required therapeutic window for the immune system to distinguish between healthy and tumour cells.

The telomerase-derived 16-mer peptide GV1001 (Pharmexa A/S) has previously been demonstrated to elicit T-cell responses in non-small cell lung cancer patients without exhibiting cytotoxicity towards
telomerase-positive bone marrow stem cells. \(^\text{18}\) GV1001 was recently subjected to a phase I/II study to investigate its safety and tolerability in patients with non-resectable pancreatic adenocarcinoma. \(^\text{19}\) A rapid and significant immune response was observed in 75% of the group receiving an intermediate dose of the peptide. There was no overall increase in immune response in the patient group receiving high peptide doses, possibly due to high local concentration of peptide leading to leakage into surrounding tissue, uptake and presentation of vaccine by non-professional antigen-presenting cells and the subsequent selection of T-cells with low-affinity T-cell receptors or anergy. Importantly, the vaccine was well-tolerated, with no serious adverse side effects or withdrawals due to treatment-related events. Furthermore, although survival was not the primary endpoint of the study, a significant correlation was observed between overall survival and immune response with a median survival of 8.6 months for patients receiving intermediate doses compared with 4.5 months for those receiving high or low doses. Accordingly, after 300 days, all surviving patients were those who had demonstrated an immune response to the peptide. These encouraging results suggest that GV1001 is immunogenic, safe and may offer a new therapeutic option for the treatment of pancreatic cancer either alone, or in conjunction with current conventional therapies. The large (>1000 patients) phase III TeloVac trial is currently ongoing. It aims to examine gemcitabine and capecitabine chemotherapy with concurrent or sequential GV1001 in patients with locally advanced and metastatic pancreatic cancers.

**Inhibition of angiogenesis**

Angiogenesis, the establishment of new vasculature, is essential during embryogenesis, wound healing and normal reproductive function in the female. \(^\text{20}\) Inappropriate angiogenesis however contributes towards several diseases such as rheumatoid arthritis, age-related macular degeneration and cancer. \(^\text{21}\) Research over the last three decades has provided a body of evidence demonstrating that the requirement for vasculature to provide oxygen, nutrients and to remove waste products at both primary and secondary sites obliges tumour cells to reside within 100–200 \(\mu\)m of a capillary blood vessel or face apoptosis and necrosis. \(^\text{9}\)

The transition between vascular quiescence and angiogenesis, known as the ‘angiogenic switch’, results from dominance of pro-angiogenic over anti-angiogenic signals and represents a significant obstacle for the growing tumour. \(^\text{22}\) The angiogenic switch is activated in tumours by the over-production of angiogenic inducers, and by neutralizing inhibitors. \(^\text{23}\) Although the development of vessel walls is an extremely
complex process, requiring a tightly choreographed series of receptor-ligand interactions and the participation of several signalling pathways, the vascular endothelial growth factor (VEGF) is generally accepted to represent a critical rate-limiting step and has thus far become a focus in the quest for anti-angiogenic agents.

The VEGF family consists of six dimeric glycoproteins: VEGF-A, -B, -C, -D, -E and placental growth factor (PGF). VEGF-A is the most extensively studied member of the family and comprises five isoforms, generated by alternative splicing of 121, 145, 165, 189 and 206 amino acids.

VEGF activity is a potent mitogen for micro- and macro-vascular endothelial cells derived from arteries, veins and lymphatics and an effective activator of angiogenesis in vivo. Additionally, VEGF is a regulator of vascular permeability, vasodilatation, endothelial migration and gene expression. VEGF-A mediates these biological effects through binding to two VEGF receptors: VEGF receptor (VEGFR)-1 and VEGFR-2.

Although pancreatic tumours are not grossly vascular, VEGF-A, VEGFR-1 and VEGFR-2 are often co-expressed implying an autocrine effect of VEGF on pancreatic cells expressing VEGF receptors, and paracrine effects on microvascular endothelial cells. Studies in animal models have shown that perturbation of the VEGF signalling pathway inhibits pancreatic tumour growth and angiogenesis. Finally, several studies have demonstrated a correlation between tumour VEGF-A levels, blood vessel density, tumour size and local metastasis of pancreatic adenocarcinoma. Taken together, these studies indicate that VEGF-A promotes tumour growth and metastasis in pancreatic cancer by encouraging angiogenesis, and thus provide a rationale for the targeting of VEGF in pancreatic cancer.

The humanized monoclonal antibody bevacizumab (Avastin®, Genentech) targets all known isoforms of VEGF-A. In preclinical studies, bevacizumab showed encouraging inhibition of tumour cell line growth in nude mice and was deemed to be non-toxic when either used alone, or in combination with standard chemotherapy in early-phase clinical trials. Based on exciting phase III results, bevacizumab, in combination with standard therapeutic regimes, has been approved by the United States Food and Drug Administration (FDA) as a first-line therapy for colorectal cancer, demonstrating that VEGF inhibition is a clinically effective strategy to treat at least one type of human cancer. Consequently, a non-randomized phase II study involving 52 patients to assess the efficacy of bevacizumab with gemcitabine as a first-line treatment for metastatic pancreatic cancer was initiated. Gemcitabine was administered at 1000 mg/m² weekly for 3 out of 4 weeks and bevacizumab at 10 mg/m² on days 1 and 15 in a 28-day treatment cycle. Results of this study demonstrated that combination therapy resulted in a superior...
objective response rate, progression-free survival (5.4 months), median survival (8.8 months) as well as 1-year survival rate (29%) when compared with gemcitabine alone, warranting further clinical assessment. The combination of gemcitabine and bevacizumab was relatively well-tolerated, although 4 out of the 52 patients developed severe gastrointestinal complications including bowel perforations and oesophageal tears. In response to these encouraging data, a phase III trial involving 602 patients receiving either gemcitabine with placebo or bevacizumab was conducted. 48 Disappointingly however, preliminary results have indicated that the combination therapy has no significant clinical benefit over gemcitabine alone.

As VEGF inhibition can overcome radioresistance, possibly by attenuating the protection afforded to endothelial cells by VEGF following radiation, 49,50 a phase I study was initiated to evaluate the safety of bevacizumab in combination with the oral fluoropyrimidine capecitabine-based chemoradiotherapy in patients with locally advanced pancreatic cancer. 7 As bevacizumab did not significantly increase the toxicity of chemoradiation therapy, further studies are justified and indeed trials are currently being conducted to investigate the benefit of bevacizumab in pancreatic cancer in a number of clinical contexts such as combination therapy with other chemotherapeutic agents and as an adjuvant therapy following surgical resection.

**Inhibition of RAS**

The 21 kDa RAS family proteins were first identified as cellular homologues of oncoproteins encoded by the genomes of the Harvey and Kirsten murine sarcoma retroviruses. Four oncogenic RAS proteins have been described thus far, HRAS, NRAS, KRAS-4A and KRAS-4B, with the two KRAS proteins arising from alternative splicing of a single gene transcript. All family members exert their biological functions by assembling signalling complexes at the cell membrane that activate intracellular signalling cascades, altered transcriptional profiles and changes in diverse cellular processes such as migration, survival, proliferation, endocytosis, differentiation, senescence and more. 51 As RAS proteins are anchored to the cell membrane and toggle between an inactive guanosine diphosphate (GDP)-bound and an active guanosine triphosphate (GTP)-bound state, they act as molecular switches in the cell, transmitting signals from the cell membrane to the nucleus. In quiescent cells, RAS is usually found in the ‘OFF’ state, until activation by upstream signalling components such as the binding of epidermal growth factor (EGF) to its cognate receptor tyrosine kinase, the EGF receptor (EGFR). Such events initiate signalling cascades resulting in
a conformational change in RAS, the disassociation of GDP and the binding of GTP, thereby switching RAS into its ‘ON’ state. In this state, RAS induces activation of distinct effector signalling cascades such as the RAF serine/threonine kinase, the phosphoinositide 3-kinase (PI3K) or the three RAL exchange proteins: RAL guanine nucleotide disassociation stimulator (RALGDS), RALGDS-like gene (RGL/RSB2) and RGL2/RLF. The signalling cascades elicited by RAS are transient and short-lived by virtue of RAS’ own intrinsic GTPase activity which is stimulated by cytosolic GTPase activating proteins (GAPs).

In tumours, RAS proteins are frequently found in the constitutively ‘ON’ states. This is often due to activating mutations in RAS genes themselves which abolish GAP-induced GTPase activity and hence render RAS unable to switch back to the ‘OFF’ state. De-regulated RAS signalling can lead to uncontrolled proliferation, enhanced migration and invasion, resistance to apoptosis and angiogenesis, and it is thus unsurprising that RAS mutations, which almost always occur at codons 12, 13 and 61, are found in about 30% of all human tumours. KRAS mutations are exceptionally frequent events in pancreatic cancers and predominantly occur at codon 12. Inhibition of RAS activity is thus an appealing anti-cancer endeavour, and several approaches have been developed to achieve this goal.

Much attention has been given to the inhibition of post-translational events that are absolutely required for RAS activation and functional specificity. Newly synthesized RAS and RHO proteins are cytosolic, and require further processing in order to associate with the lipid membrane. A pivotal step in this pathway is the covalent addition of a hydrophobic 15-carbon farnesyl to the cysteine residue within the CAAX motif (C, cysteine; A, any aliphatic amino acid; X, typically methionine or serine) at the extreme C-terminus of the protein. This event, known as farnesylation, is mediated by farnesyltransferase (FT). The -AAX amino acids are cleaved off by RAS-converting enzyme I, and the farnesylated cysteine is carboxymethylated by isoprenylcysteine carboxy methyl transferase. Finally, HRAS, NRAS and KRAS-4A are palmitoylated by palmitoyltransferase, which facilitates the addition of two long-chain fatty-acid groups to their C-terminal cysteine residue and anchors the proteins to the cell membrane. KRAS-4B is not a substrate for palmitoylation but its interaction with the cell membrane is stabilized by the presence of a polybasic domain. The absolute requirement for these modifications was demonstrated by the fact that mutant RAS proteins which lack the isoprenylated cysteine could not associate with the membrane and could not transform fibroblasts. These results prompted the search for farnesyltransferase inhibitors (FTIs) for use as anti-cancer agents. The first generation FTIs were largely peptides designed to mimic the CAAX motif and thus
compete for binding to farnesytransferase (CAAX peptidomimetics). Newer compounds were designed to compete with the farnesyl pyrophosphate or share characteristics of both farnesyl pyrophosphate and the CAAX motif (bisubstrate analogues). Given the necessity for RAS signalling in normal growth factor signalling and proliferation, it was somewhat surprising that FTIs displayed negligible toxicity and did not appear to interfere with normal cell proliferation in early in vitro studies. Preclinical studies using these compounds produced very encouraging data and FTIs demonstrated impressive anti-proliferative effects against several different tumour cell lines including those of pancreatic origin. Moreover, the FTI R115777 (tipifarnib/Zarnestra®, Johnson and Johnson Pharmaceutical Research and Development) demonstrated anti-proliferative effects in pancreatic cancer cell lines at clinically relevant concentrations, and also showed significant tumour growth inhibition and anti-angiogenic activity in a pancreatic xenograft model. Critically, the CAAX analogue L-744,832 potently reversed mammary tumour development in transgenic mice expressing oncogenic H-Ras under the control of the MMTV promoter. FTIs also showed promise in early-phase clinical trials. Prolonged administration of single-agent R115777 was deemed to be non-toxic and well-tolerated. However, these promising results were not recapitulated in later-stage trials. R115777 was found to be ineffective as first-line monotherapy for advanced pancreatic cancer. The phase II study, conducted by the South West Oncology Group, reported that administration of R115777 resulted in a median time to progression of 1.4 months and a median survival of 2.6 months. As these data were inferior to those obtained with gemcitabine, the study concluded that R115777 was not an active agent in pancreatic cancer.

A recent phase III study involving 688 patients also deemed R115777 ineffective when used in combination with gemcitabine for advanced pancreatic cancer. Although the combination of R115777 and gemcitabine was well-tolerated, no statistically significant improvement in overall survival was observed when compared with gemcitabine monotherapy (median survival of 193 versus 182 days, respectively).

The unimpressive clinical trial data of FTIs may in part be attributed to the fact that KRAS and NRAS proteins are alternatively prenylated by the addition of a 20-carbon geranylgeranyl isoprenoid moiety by geranylgeranyltransferase I (GGT). Given that the majority of RAS mutations in human cancers occur in the KRAS gene, the lack of toxicity and clinical efficacy of FTIs is thus unsurprising. Subsequent preclinical studies of concomitant FT and GGT inhibition have demonstrated efficacy in pancreatic cancer cell lines, but revealed high levels of toxicity. In spite of this, a phase I study of the dual FT and GGT inhibitor L-778,123 in combination with radiotherapy was conducted...
in patients with locally advanced pancreatic cancer. Although the combination of radiation and L-778,123 displayed acceptable toxicity at low doses, further development of this compound has been halted due to adverse cardiac effects observed in other studies. Despite their debatable mode of action, and their largely disappointing results in clinical trials to date, FTIs have shown some early potential in the treatment of leukaemias. If shown to be useful, at least in certain clinical settings, FTIs will not only benefit patients, but will also strengthen the premise that targeting signalling molecules represents an exciting strategy for future cancer drug development. Clearly, further work is needed to ascertain whether this will indeed be the case.

A further strategy to inhibit RAS function has been through the use of antisense technology. This method utilizes antisense oligonucleotides which are typically 13–25 nucleotides long and have been designed to hybridize with the target mRNA. Subsequent gene silencing occurs when protein translation is blocked by RNAse H-mediated cleavage of the mRNA-oligonucleotide duplex. Despite the predominance of KRAS mutations in pancreatic cancer, the selective targeting of HRAS has also been explored as a therapeutic option. ISIS-2503 is a 20-base antisense oligonucleotide that prevents translation initiation of HRAS mRNA. In vitro studies have shown ISIS-2503 to be a potent and selective inhibitor of HRAS, which, in a phase I study, was deemed to be safe and non-toxic when combined with gemcitabine and used against advanced pancreatic cancer. This was followed by a phase II study involving 48 patients with locally advanced or metastatic disease. ISIS-2503 combined with gemcitabine produced a response rate of 10.4%, including one complete response, and median survival of 6.6 months. In agreement with the phase I data, the combination treatment was relatively well-tolerated with an acceptable toxicity profile. Given that these results are superior to those obtained by FTI inhibition, this antisense technology currently represents the RAS-targeting strategy with the most promise.

**MEK inhibition**

The mitogen-activated extracellular kinase (MEK) is a principal component of the RAS-RAF-MEK-extracellular signal-regulated kinase (ERK) which plays a core role in several cellular processes including proliferation, apoptosis, differentiation and angiogenesis. The pathway is also known to be de-regulated in a number of cancers including those of the breast, lung, colon and pancreas, and thus represents an appealing target for the development of novel therapies.

CI-1040 is a low-molecular weight inhibitor of MEK1/2 which demonstrated impressive anti-proliferative activity against pancreatic
cancer xenografts. Unsurprisingly, the efficacy of CI-1040 is dependent on constitutive activation of the MEK–ERK pathway, making pancreatic cancer an ideal clinical setting for application of CI-1040 due to the high frequency of RAS mutations. In a phase I study of 77 patients with advanced malignancies including those of colorectal, lung, kidney, skin and pancreas, CI-1040 was well-tolerated with a satisfactory safety profile. The most common treatment-associated toxicities were low-grade diarrhoea, acneiform rash, nausea and vomiting. Even more encouraging was the fact that one pancreatic cancer patient demonstrated a partial response, with improvements in pain, fatigue and anorexia. Testing of CI-1040 progressed to a phase II study of 67 patients with advanced colorectal, lung, breast or pancreatic cancer. In this setting, CI-1040 failed to demonstrate sufficient antitumoral activity and development was thus discontinued.

Inhibition of RAF has also been investigated in the context of pancreatic cancer. The small molecule MEK inhibitor sorafenib (BAY 43-9006, Bayer) also inhibits VEGFR-2, VEGFR-3 and platelet-derived growth factor receptor-β, and was tested in a phase II trial for the treatment of advanced solid tumours in combination with gemcitabine. The combination of 400 mg sorafenib bi-daily with 1000 mg/m² gemcitabine was found to be relatively well-tolerated and 13 pancreatic cancer patients (56.5%) showed evidence of disease stabilization. However, a more recent phase II trial of 17 patients failed to show any objective response in patients with advanced pancreatic cancer. A phase III trial is currently in progress.

**EGFR inhibition**

The EGFR is a member of the ErbB family of transmembrane receptor tyrosine kinases which comprises four members: ErbB1/HER1/EGFR, ErbB2/HER2/neu, ErbB3/HER3 and ErbB4/HER4. Structurally, ErbB receptors consist of an extracellular ligand-binding domain, a transmembrane domain and an intracellular protein kinase domain containing a regulatory carboxyl terminal segment. Engagement of receptor with cognate ligands such as EGF, transforming growth factor-alpha (TGF-α), heparin-binding growth factor, amphiregulin, betacellulin or epiregulin results in homo- or hetero-dimerization among the ErbB family, leading to activation of intrinsic protein kinase activity, followed by tyrosine trans-auto-phosphorylation, accompanied by the recruitment of intracellular protein substrates. Phosphorylated tyrosine residues on the receptor also create binding sites for various adaptor and docking proteins.

Receptor tyrosine kinases play pivotal roles in the regulation of a number of cellular processes such as survival, proliferation, migration,
apoptosis, invasion and metastasis. The first indication of a role for the EGFR gene in cellular transformation came from the finding that EGFR is the cellular homologue of the avian erythroblastosis virus v-erbB oncogene. Since this landmark discovery, several studies have shown that inappropriate EGFR signalling arising from gene amplification, receptor or ligand over-expression, activating mutations or inactivation of negative regulatory pathways, is a feature of several human tumour types including pancreatic cancer. Co-expression of EGFR (found in 69% of pancreatic adenocarcinoma) and its ligands is frequently observed in pancreatic cancer and functions as an autocrine loop, constitutively stimulating pancreatic cancer cell proliferation. A correlation between co-expression of EGFR and TGF-α with increased tumour size, advanced clinical stage and poor prognosis has been reported, and thus may represent a molecular signature of a more aggressive disease. Studies have also suggested an association between EGFR expression and metastasis, particularly to the liver. Moreover, EGFR and TGF-α have also been implicated in tumour angiogenesis by virtue of their ability to induce VEGF secretion.

Taken together, these data suggest that attenuation of the EGFR pathway may not only block tumour cell proliferation, but may also inhibit both angiogenesis and metastasis. In the first preclinical studies of EGFR inhibition, a panel of monoclonal antibodies directed against the EGF-binding site on EGFR inhibited the growth of cancer cells expressing high levels of EGFR in both cell culture and xenograft models. These data were the first to demonstrate a potent antiproliferative effect of EGFR inhibition and paved the way for a serious effort to develop EGFR inhibitors for use as anti-cancer agents. To date, two main classes of EGFR inhibitors have been developed, the first being small molecule inhibitors which interfere with adenosine triphosphate (ATP) binding and the second being monoclonal antibodies which prevent association with ligands.

Cetuximab (IMC-C225/Erbitux®, ImClone Systems/Merck KGaA) is a chimeric human–mouse monoclonal antibody directed against EGFR. In May 2004, cetuximab received FDA approval for the treatment of irinotecan-resistant or -intolerant colorectal cancer. Cetuximab has been tested against pancreatic cancer in a number of preclinical settings, both as single-agent monotherapy and in combination with gemcitabine. Cetuximab potently inhibited EGFR auto-phosphorylation in human pancreatic cancer cell lines and orthotopic tumour models. The observed anti-proliferative effect of cetuximab alone was modest (only 20%), but was enhanced upon addition of gemcitabine. In the same study of murine orthotopic pancreatic tumour models, cetuximab reduced tumour volume and liver metastasis either alone or with gemcitabine. Systemic administration of cetuximab also resulted in decreased
VEGF and interleukin-8 (IL-8) expression, accompanied by increased apoptosis in endothelial cells and a concomitant decrease in microvessel density, suggesting that cetuximab was also exerting an anti-angiogenic effect. Other groups have reported similar findings. These results prompted a phase II trial to evaluate the efficacy of cetuximab in combination with gemcitabine in locally advanced and recurrent pancreatic cancers. Patients received weekly 1000 mg/m² gemcitabine initially for 7 out of 8 weeks and 3 out of 4 weeks thereafter. Cetuximab was administered at a loading dose of 400 mg/m² followed by weekly infusions of 250 mg/m².

In keeping with the results of previous studies, cetuximab was well-tolerated with the most common grade 3 and 4 adverse events being neutropaenia, abdominal pain and asthenia. In agreement with previous data, patients also developed an acne-like rash, which was only severe in 12% of cases, and has been attributed to the inhibition of EGFR in the skin. Significantly, the median survival was 7.1 months and 31.7% of patients were alive after one year, suggesting that the combination of gemcitabine and cetuximab may be more effective than gemcitabine monotherapy. These encouraging results led the design of two further trials. The first, conducted by the Southwest Oncology Group, was a phase III trial to study cetuximab alone or in combination with gemcitabine as first-line treatment for advanced pancreatic cancer. Disappointingly however, this study failed to show any significant benefit of cetuximab addition for response, progression-free survival and overall survival. The second study, conducted by the Eastern Cooperative Oncology Group, is currently ongoing and aims to investigate the combination of docetaxel and irinotecan with or without cetuximab.

Matuzumab (EMD72000, Merck KGaA) is also a humanized monoclonal antibody, which binds EGFR with high specificity and affinity. Preclinical trials using orthotopic tumour models showed that matuzumab administration resulted in significantly decreased tumour progression and angiogenesis. A subsequent phase I trial was launched to assess the safety and benefit of using matuzumab in combination with gemcitabine as a first-line treatment for advanced pancreatic cancer. Results of this study demonstrated that the combination was relatively well-tolerated at the biologically active dose of 800 mg matuzumab per week, with stable disease observed in 65% of patients. Further clinical trials with this compound are being planned.

Trastuzumab (Herceptin, Genentech) is a recombinant humanized antibody against ErbB2 (HER2/neu) and received FDA approval for breast cancer treatment in 1998. Encouraged by the success of trastuzumab in the treatment of breast cancer, researchers began to explore the benefit of using this compound for the treatment of other malignancies,
including pancreatic cancer where ErbB2 is over-expressed in about 50% of cases.\textsuperscript{107,108} However, in a phase II clinical trial the combination of gemcitabine and trastuzumab was not significantly superior to gemcitabine alone.\textsuperscript{109} A second trial to study trastuzumab in combination with chemoradiation has recently been completed and results are awaited.

Small molecule inhibitors of EGFR were developed on the basis of the observation that mutation of the ATP-binding site of EGFR potently attenuated its receptor tyrosine kinase activity.\textsuperscript{110} These findings led to the large-scale screening of compound libraries for molecules able to compete for the ATP-binding pocket of the tyrosine kinase domain of EGFR. One such molecule is erlotinib (OSI-774/Tarceva\textsuperscript{®}, Genentech) which received FDA approval in 2004 for the treatment of patients with advanced non-small cell lung cancer, and is being increasingly tested for use in other cancers including colorectal, ovarian and pancreatic cancers.

In a recently published, landmark phase III trial, 569 patients with advanced pancreatic cancer were randomized to receive gemcitabine (1000 mg/m\textsuperscript{2}/week for 7 out of 8 weeks) with placebo or 100 mg erlotinib daily, although a small cohort of patient received a higher dose of 150 mg.\textsuperscript{111} The results of this study demonstrated that erlotinib and gemcitabine combination therapy resulted in a small but statistically significant increase in median survival (6.24 versus 5.91 months, respectively) and 1-year overall survival (23 versus 17%). Response rates and quality of life analysis gave similar results for both patient groups. Importantly, the combination of gemcitabine and erlotinib was well-tolerated with diarrhoea and rash being the most commonly observed toxicities associated with the addition of erlotinib. Interestingly, patients with grade 2 or higher skin rash experienced statistically significant survival. This study was the first to show a statistically significant benefit of combination therapy over gemcitabine monotherapy, and consequently, erlotinib became the first targeted therapy approved by the FDA for use in pancreatic cancer in 2005. Recently however, Grubbs et al.\textsuperscript{112} have questioned whether this combination is in fact cost-effective. Although the increase in median survival gained by the addition of erlotinib to gemcitabine was statistically significant, it was modest (an increase of 0.4 months over gemcitabine alone), and is therefore not cost-effective even at the highest year-per life gained parameters. Thus, the overall clinical impact of erlotinib with gemcitabine therapy on the treatment of pancreatic cancer remains to be seen.

In addition, erlotinib has been investigated in combination with capecitabine in gemcitabine-refractory advanced pancreatic cancer.\textsuperscript{113} Patients were administered 1000 mg/m\textsuperscript{2} capecitabine twice daily for 14 out of 21 days, and 150 mg erlotinib daily. The regimen was
well-tolerated with rash and diarrhoea being the most frequently observed toxicities. Given that the study reported an overall response rate of 10% and a median survival of 6.5 months, it was concluded that this combination may represent an appropriate treatment for patients for whom gemcitabine is either ineffective or inappropriate, and further analysis is required to establish whether this is indeed the case.

Preclinical studies have revealed a role for erlotinib as a potent radiosensitizing agent.\textsuperscript{114} Thus, the feasibility of using the novel combination of erlotinib, gemcitabine, paclitaxel and concomitant radiotherapy followed by maintenance erlotinib for locally advanced pancreatic cancer was the subject of a phase I trial conducted by Iannitti et al.\textsuperscript{115} Here, the maximum tolerated dose of erlotinib during chemoradiation therapy was 50 mg daily due to dose-limiting diarrhoea. Given that this trial reported an encouraging partial response rate of 46% and median survival of 14 months, this treatment regimen merits further investigation.

Gefitinib (ZD1839/Iressa,\textsuperscript{w} AstraZeneca) is a low molecular weight EGFR inhibitor. It was the first tyrosine kinase inhibitor to receive FDA approval for the treatment of advanced lung cancer in 2003, and is currently under investigation as treatment for several other tumour types including pancreatic cancer. In early-phase clinical trials, gefitinib used in combination with capecitabine and radiation therapy resulted in significant toxicity even at the lowest administered doses.\textsuperscript{116} A phase II trial of gefitinib and gemcitabine in patients with inoperable or metastatic pancreatic cancer has shown results similar to those of erlotinib with gemcitabine.\textsuperscript{117} However, results from phase II trials of gefitinib and docetaxel as second-line therapy were unremarkable.\textsuperscript{118,119}

### Gastrin inhibition

The peptide hormone gastrin is produced in large amounts by G cells of the gastric antrum, and in smaller amounts by the upper small intestine, colon and pancreas, and mediates the secretion of pancreatic juice.\textsuperscript{120}

Expression of cholecystokinin (CCK)/gastrin receptor and the gastrin precursors progastrin, glycine-extended gastrin (Ggly) and amidated gastrin (Gamide) has been observed by immunohistochemistry in 95, 91, 55 and 23% of pancreatic tumours, respectively.\textsuperscript{121} Gastrin stimulates the proliferation of pancreatic carcinoma\textsuperscript{122} which, conversely can be blocked by the administration of antisense oligonucleotides to gastrin.\textsuperscript{123} Further experimentation revealed that the over-expression of CCK-2 in transgenic mice increased pancreatic weight by 40% and resulted in a 15% rate of malignant transformation when these mice
were crossed with mice expressing Gamide in pancreatic β-cells. Together, these data provided the rationale for the development of anti-gastrin therapy to treat pancreatic cancer.

The gastrin receptor antagonist gastrazole (JB95008, James Black Foundation) was recently the subject of two trials involving pancreatic cancer. The first study recruited 18 patients and compared gastrazole with placebo. Overall, gastrazole resulted in a significant improvement in median survival (7.9 versus 4.5 months) and 1-year survival (33 versus 11%), while displaying minimal toxicity. The second study compared gastrazole with 5-FU but failed to demonstrate meaningful difference between the two patient groups, although gastrazole was associated with fewer toxicities.

An alternative strategy is the use of immunogens to stimulate the production of neutralizing antibodies against the serum- and tumour-associated forms of gastrin-17. One such immunogen is G17DT (gastrimmune/Insegia®, Aphton Corporation) which is administered by intramuscular injection and was the subject of a phase II trial with 30 patients with advanced pancreatic cancer. While the majority (82.4%) of patients mounted an antibody response to the immunogen, G17DT resulted in severe adverse reaction in three patients, although interestingly these patients survived for longer than the median survival of the group (244 days). Furthermore, the median survival of responders (217 days) was significantly longer than that of non-responders (121 days) indicating that G17DT does in fact possess anti-tumoral properties. Nevertheless, a subsequent phase III trial of G17DT in combination with gemcitabine failed to improve overall survival or secondary endpoints (e.g. response rate, time to progression).

Proteinase inhibition

The aggressive nature of pancreatic cancer is partly attributed to its early and frequent invasion of local structures, neural invasion and metastasis to the liver and lymph nodes, all of which preclude surgical resection and contribute towards the lethality of the disease. Metastasis, which is a multi-step process during which cells within a primary tumour disengage and migrate to secondary organs, can only occur once cells are endowed with the ability to escape the primary tumour, invade surrounding tissue, enter and exit blood vessels and extravasate and establish metastatic foci at secondary sites. During the metastatic process, cells require the ability to degrade their surrounding matrix.

The matrix metalloproteinases (MMPs) are a family of zinc-dependent proteolytic enzymes that are involved in the degradation of
the extracellular matrix (ECM). The family can broadly be subclassified on the basis of substrate specificity: collagenases (MMP-1, -8 and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -7, -10) membrane types (MMP-14/MT-MMP1, MMP-15/MT-MMP2, MMP-16/MT-MMP3, MMP-17/MT-MMP4, MMP-24/MT-MMP5) and others (MMP-18, MMP-19, MMP-20/enamelysin). Based on this ability to destroy the ECM, MMPs were immediately implicated in tumour progression and a strong association between MMP expression patterns with tumour progression is now widely accepted.133 Studies in pancreatic cancer have shown that MMP-2, -7, -8, -9 and -11 are overexpressed in malignant tissue, with high MMP-7 levels correlating with shortened survival.134,135 Of note, MMP-7 expression is also increased during metaplastic transition in a murine model of pancreatic acinar-to-ductal metaplasia.136 Taken together, these observations supported the development of MMP inhibitors (MPIs) for use as therapeutic agents in pancreatic cancer.

Preclinical results for MPIs were encouraging. The MPI SC44463 proved to be effective in blocking metastasis in an early mouse model.137 Moreover, in orthotopic murine models of pancreatic cancer, administration of the MPI batimastat (BB-94, British Biotech) reduced metastases and also significantly prolonged overall survival with or without gemcitabine therapy.138,139 Unfortunately clinical trials with MPIs have not lived up to the initial high expectations. Of particular concern was the fact that one trial of the MPI tanomastat (BAY 12-9566, Bayer) in patients with advanced pancreatic cancer was terminated early as patients receiving tanomastat showed significantly poorer survival than those receiving placebo.140 However, these negative effects on survival were not recapitulated in subsequent studies of another MPI, marimastat (BB2516, British Biotech), indicating that the adverse effects of tanomastat were probably mediated by an as yet unclear mechanism. Marimastat has in fact been the subject of several clinical trials in pancreatic cancer patients. Early-phase trials demonstrated that marimastat administration was associated with a decrease or stabilization in pain, mobility and analgesia scores in 51% of patients and was relatively well-tolerated, with the major toxicity being musculoskeletal.141 Based on these findings, two randomized phase III trials were conducted with the aim of comparing marimastat with gemcitabine as first-line therapy for patients with unresectable pancreatic cancer. A total of 414 patients received marimastat (5, 10 or 25 mg twice daily) or 1000 mg/m² gemcitabine.142 No significant difference in 1-year survival was observed between patients receiving marimastat and those on the standard gemcitabine therapy. Furthermore, a subsequent study failed to show any significant clinical benefit of using marimastat in combination with gemcitabine over gemcitabine monotherapy.143
It has however been argued that the failure of marimastat and other MPIs to demonstrate clinical efficacy in advanced pancreatic cancer may be attributed to an inherent flaw in design of the clinical trials. Critics argue that trials of marimastat have included large numbers of patients with metastatic disease, yet the cytostatic nature of these agents suggests that MPIs decrease the rate of tumour progression, and this would be of maximum therapeutic benefit in early stages of the disease. In agreement with this notion, it has been noted in pancreatic cancer patients that marimastat increased overall survival time in patients without overt metastatic disease compared with those with metastases at trial entry.

A further problem is the failure of phase II trials to define adequately the biologically active dose of MPIs, due to the lack of a suitable endpoint measurement. As MPIs are cytostatic and not cytotoxic agents, reduction in tumour size is unlikely and therefore an inappropriate parameter. Several studies have used changes in serum concentrations of the tumour antigen CA 19-9 as a measure of MPI efficacy, but has attracted criticisms, as alterations in biomarker levels do not always represent tumour regression. Supporters of MPI therefore maintain that the true potential of these compounds can only be fairly assessed once these limitations have been addressed. Until then, the role of MMP inhibition in the clinic remains uncertain.

**COX-2 inhibition**

An association between inflammation and cancer has been demonstrated by several epidemiological studies although the molecular nature of this relationship is yet to be completely understood. It is known however that mediators of the inflammatory process such as growth factors, cytokines and chemokines can contribute towards the development and progression of tumours.

The last decade of research has highlighted the importance of cyclooxygenases (COXs) in cancer. Two isoforms have been identified thus far; COX-1, which is required for the supply of prostaglandins to organs and COX-2, which is dramatically up-regulated following inflammation and exposure to cytokines, tumour promoters and growth factors. COX-2 plays a principal role in the metabolism of arachidonic acid to prostaglandins and thromboxanes, and thus mediates resistance to apoptosis, adhesion, motility, immunosuppression and angiogenesis. Several lines of evidence have implicated COX-2, but not COX-1 in pancreatic cancer including the observation that its over-expression at both mRNA and protein levels has been observed in both pancreatic cancer cell lines and primary tumours,
with one study finding COX-2 protein expression in 90% of pancreatic adenocarcinoma cases.\textsuperscript{148–150} Significantly, inhibition of COX-2 activity by non-steroidal anti-inflammatory drugs and COX-2-specific inhibitors such as celecoxib and rofecoxib all exert anti-proliferative effects on pancreatic cancer cell lines \textit{in vitro} and \textit{in vivo},\textsuperscript{149,151,152} and a recently published study showed that administration of the COX-2 inhibitor nimesulide delayed pancreatic intraepithelial neoplasia progression in a murine model of pancreatic cancer.\textsuperscript{153} Taken together, these data suggested that COX-2 inhibition represents a rational strategy for the treatment of pancreatic cancer. In a phase I study of patients with locally advanced disease, celecoxib (Celebrex\textsuperscript{®}, Pfizer) increased the efficacy of a gemcitabine-radiation regimen, but significantly augmented its toxicity.\textsuperscript{154} Another study reported minimal toxicities, but failed to demonstrate any clinical benefit when combining celecoxib with 5-FU in gemcitabine-refractory disease.\textsuperscript{155} Similarly, celecoxib failed to increase the sensitivity of tumours to the chemotherapy combination of cisplatin and gemcitabine.\textsuperscript{156} However, other studies yielded more promising data. Preliminary results from a phase II trial using celecoxib and gemcitabine reported a median survival of 6.2 months, 3-month survival rate of 72% and an acceptable toxicity profile.\textsuperscript{103} A second phase II trial involving 42 patients with locally advanced or metastatic disease found that the combination of gemcitabine and celecoxib (400 mg bi-daily) produced an encouraging median survival rate of 9.1 months, with an overall clinical benefit response of 54.7%.\textsuperscript{157} Overall, the treatment was well-tolerated, with renal toxicity, heartburn and anaemia (all grade 1) being the most common adverse events associated with celecoxib. Importantly however, no dose-limiting toxicities were observed. Celecoxib has also been tested in combination with gemcitabine and irinotecan, resulting in a median survival of 13 months, an 8-month median time to progression and a 1-year overall survival of 64%.\textsuperscript{158} Additionally, 76% of patients reported an improvement on overall quality of life.

Taken together, these data suggest that this class of inhibitors does display biological activity against pancreatic cancer and thus warrants further clinical investigation.

\textbf{Novel targets}

Novel drugs against newly identified targets are slowly entering the clinical trial pipeline following exciting preclinical evaluation. Taking centre stage are inhibitors of the PI3K/AKT and NFκB pathways which are emerging as critical to chemoresistance in pancreatic cancer.
The serine/threonine kinase AKT is up-regulated through gene amplification in several tumour types including pancreatic cancer.\textsuperscript{159,160} De-regulation of the AKT pathway can also be achieved through inactivation of the lipid phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome 10), which is also frequently observed in pancreatic cancer cell lines and tumours.\textsuperscript{161} The requirement of the AKT pathway in pancreatic cancer is demonstrated by the observation that its inhibition abrogates pancreatic cancer cell proliferation, induces apoptosis and restores gemcitabine-mediated chemosensitivity in several, but not all pancreatic cancer cell lines.\textsuperscript{162–165} Thus, AKT inhibition may represent an exciting therapeutic strategy, although no specific compounds have commenced clinical development thus far.

The mammalian target of rapamycin (mTOR) lies downstream of AKT and regulates cell growth by exerting effects on protein synthesis, metabolism, autophagy and ribosome biogenesis.\textsuperscript{166} An investigation of surgically resected tumours showed detectable levels of activated mTOR and its downstream kinase S6K1 in 55 and 65\% of samples, respectively,\textsuperscript{167} providing the rationale for mTOR inhibition as a therapeutic avenue for pancreatic cancer. In preclinical studies, the mTOR inhibitor CCI-779 (temsirolimus/Torisel®, Wyeth) suppressed proliferation and induced apoptosis in pancreatic cancer cell lines, and also enhanced gemcitabine-dependent apoptosis in xenograft models.\textsuperscript{167,168}

Studies have also shown that rapamycin (sirolimus/Rapamune®, Wyeth) exhibits potent anti-proliferative activity by blocking not only mTOR, but also the hypoxia-inducible factor 1α and VEGF,\textsuperscript{169,170} and was reported to exert significant anti-angiogenic and anti-proliferative effects on orthotopic tumours when used alone, and resulted in profound growth inhibition of tumour growth and local metastasis when combined with gemcitabine.\textsuperscript{171} Similarly, inhibition of mTOR in pancreatic cancer cell lines with RAD001 (everolimus/Certican®, Novartis) suppressed proliferation, induced apoptosis and sensitized cells to gemcitabine.\textsuperscript{172,173} Based on these promising results, early-stage clinical trials of mTOR inhibitors for pancreatic cancer treatment are currently in progress and their results are eagerly anticipated.

Under normal conditions, the principal function of the NFκB transcription factor is to regulate immune and inflammatory responses, although it has become a key target for therapeutic intervention due to its role in promoting proliferation, survival and chemoresistance. De-regulated NFκB expression has been reported in pancreatic tumours and cancer cell lines, but has not been observed in immortalized, non-tumorigenic pancreatic epithelial cells.\textsuperscript{174} Indeed, it has been proposed that the differential sensitivities of pancreatic cancer cell lines to gemcitabine may in fact be attributed to the level of NFκB activation, as NFκB inhibition restored chemosensitivity.\textsuperscript{175,176} At present however,
no specific NFκB inhibitors have been developed, although the proteosome antagonist bortezomib (PS-341/Velcade®, Millennium Pharmaceuticals) blocks NFκB activity by preventing degradation of its regulator IκBα and has been granted FDA approval for its use in refractory multiple myeloma. In vitro and in vivo studies have demonstrated that bortezomib significantly inhibited tumour growth of pancreatic cancer xenografts in athymic nude mice and displayed impressive anti-proliferative effects on some, but not all, pancreatic cancer cell lines. It also acts as an efficient chemosensitizer, although sensitivity does not necessarily correlate with the level of NFκB activation. Nevertheless, bortezomib performed well in phase I trials in patients with advanced solid tumours when combined with gemcitabine, displaying an acceptable toxicity profile and subsequently quickly progressed to a phase II study. Unexpectedly, this study failed to demonstrate superiority of bortezomib alone or in combination with gemcitabine compared with gemcitabine monotherapy in patients with advanced pancreatic cancer, and resulted in an unacceptable toxicity profile. It has thus been concluded that this compound is not suitable for use in pancreatic cancer treatment.

Curcumin, a phytochemical derivative of the Curcuma longa plant is an inhibitor of several molecules involved in proliferation, but also blocks NFκB-dependent transcription and subsequent activation of target genes such as COX-2 and IL-8. Administration of curcumin results in growth inhibition and apoptosis in pancreatic cancer cells in vitro, and can also potentiate gemcitabine-dependent apoptosis in cancer cell lines and in xenograft models. These data provided the rationale for a phase II trial in pancreatic cancer patients. Curcumin was relatively well-tolerated and in spite of low bioavailability, a down-regulation of NFκB, COX-2 and phosphorylated STAT3 was observed in patients’ peripheral blood. It was thus concluded that curcumin is safe and biologically active, and further trials are currently ongoing.

Other novel targets that are the subjects of intense research include the Hedgehog and Notch signalling pathways, insulin-like growth factor-1 receptor (IGF-1R), TGF-β, mesothelin and MUC1 (mucin 1, cell surface associated). Physiologically, Hedgehog and Notch signalling is important in organ development, but abnormal expression and activation could lead to pancreatic tumourigenesis. Although still in the early developmental stages, treatments such as the Hedgehog pathway inhibitor cyclopamine has shown promising results in vivo. IGF-1R has anti-apoptotic and growth-promoting effects, and is found in 64% of pancreatic cancer. Its inhibition by the AMG-479 antibody was found to enhance the anti-tumour effects of gemcitabine and erlotinib in xenograft models. TGF-β signalling is tumour
suppressive in epithelial cells, but it can promote invasion and metastasis during the later stages of cancer progression. Early data from an ongoing phase I/II study using AP 12009, an antisense oligonucleotide specific for TGF-β2 in patients with pancreatic carcinoma, malignant melanoma and colorectal carcinoma, has been encouraging. The cell membrane proteins mesothelin and MUC1 are over-expressed in the majority of pancreatic cancer. They have been studied as targets for immunotherapy, but further research is still required.

**Outlook and future perspectives**

The failure of conventional chemotherapeutic regimes to produce any meaningful impact on survival in patients with pancreatic cancer highlights a desperate need for novel treatment strategies. New-generation, targeted drugs offered an obvious new avenue for hope, owing to their successes in the treatment of chronic myeloid leukaemia and cancers of the breast and colon. However, despite numerous clinical trials involving thousands of patients, these drugs have yet to revolutionize the treatment of advanced pancreatic cancer.

The advent of molecular medicine has transformed the ways in which cancer is approached both clinically and intellectually. Technologies such as high-throughput gene expression profiling using microarray analysis have allowed investigators to identify genes whose expression patterns correlate with drug sensitivity, disease staging and prognosis, as well as molecular targets against which novel drugs may be directed, offering clinicians a greater arsenal of treatment options. This, in the case of several malignancies, has translated successfully into the clinic. The routine genetic profiling of tumour samples for key indicator genes such as HER2/neu in breast cancer provides not only important prognostic information, but also guidance as to whether a patient will respond to a particular treatment regime. Thus, an individualized treatment plan can be tailored to suit the specific profile of the patient. Trastuzumab has transformed the bleak landscape of breast cancer for thousands of women and provides an excellent example of how the successful integration of targeted therapies and tumour profiling can be when applied to the appropriate clinical context. The development of both trastuzumab and bevacizumab also illustrates the importance of appropriate patient selection into clinical trials, as indiscriminate recruitment may potentially mask the efficacy of a particular drug. Tumours are heterogenous and complex, making it extremely unlikely that all tumours within a particular subtype will harbour the same activating mutation, or indeed that the tumour will be entirely dependent on the de-regulation of one particular pathway. Thus, while the pursuit
of novel targeted agents is mandatory, this must be coupled with the development of new strategies to allow us to identify patients who will most benefit from these drugs.

Herein perhaps lies the reason behind the failure of targeted therapies to provide any meaningful impact on the management of pancreatic cancer. While our knowledge of the molecular characteristics of this disease has risen exponentially over the past decade, we have been less successful in applying this information in a clinical context. This may in part be due to the unique nature of pancreatic cancer where the majority of patients present with inoperable disease, and tissue samples obtained for diagnosis do not provide enough material for profiling techniques outlined above. This profound lack of tumour material has precluded the retrospective identification of biomarkers associated with clinical response to a particular drug, and the subsequent selection of appropriate patients into a clinical trial, as well as the limited identification of a gene-expression pattern present in individuals predisposed to the disease. The development of improved methods to readily obtain tumour material sufficient for genetic analyses must therefore be a high priority.

Future trials may also benefit from a shift away from conventional chemotherapy combinations and instead focus on combining targeted therapies. Some studies have been performed on the basis of this hypothesis, both combining EGFR and VEGF inhibitors. A phase II trial, conducted by the University of Chicago, aimed to evaluate gemcitabine and bevacizumab in combination with either erlotinib or cetuximab in 139 patients with advanced pancreatic cancer. Results, however, were far from impressive, although there was a trend for improved overall survival in patients who developed grade 2 or higher skin rash after treatment with gemcitabine, bevacizumab and erlotinib. The AVITA/BO17706 trial, sponsored by Roche, is a randomized, double-blind, placebo-controlled phase III study examining the benefit of adding bevacizumab to gemcitabine and erlotinib in 607 patients with metastatic pancreatic cancer. Preliminary results indicated that the addition of bevacizumab did not significantly prolong overall survival, but there was a trend towards improved survival. Significant improvement in progression-free survival was noted. A recent phase II study of bevacizumab and erlotinib in 26 patients with gemcitabine-refractory metastatic pancreatic cancer has demonstrated modest benefit.

Despite these failures, our collective effort has not only created a substantial platform of knowledge from which future studies can spring, but has also moulded a robust clinical trial framework which will allow us to better address these issues in the future. It is hoped that the next decade will bring improved diagnostic tools and newer, more effective therapies to patients who will benefit the most. Together, this may finally allow us to improve the currently gloomy outlook for pancreatic cancer patients.
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