New target therapies in advanced pancreatic cancer

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The recent elucidation both of the mechanisms involved in pancreatic cancer carcinogenesis and the related molecular events, has led to several distinct therapeutic advances, including many novel target agents, such as monoclonal antibodies against EGFR, EGFR-tyrosine kinase inhibitors, monoclonal antibody against VEGF, farnesyl transferase inhibitors, matrix metalloproteinase inhibitors, COX 2 inhibitors, and the development of gene therapy to target pancreatic cancer. This review highlights recent findings in the treatment of pancreatic cancer by using these novel therapeutic approaches.

Key words: pancreatic cancer, EGFR inhibitor, farnesyl transferase inhibitor, COX-2 inhibitor

Pancreatic cancer is the fourth leading cause of cancer deaths in the United States and the sixth in Europe. In 2000 it was estimated that 213,000 men and women would die of pancreatic cancer worldwide [1]. The overall 5-year survival rate for patients with pancreatic cancer ranges from 1% to less than 5%, and there has been little improvement in survival rates in the last 20 years. Therapeutic options for advanced pancreatic cancer are limited. Patients presenting with locally advanced pancreatic cancer are either treated with chemoradiotherapy, generally a fluorouracil or gemcitabine based regimen, or with gemcitabine alone. For tumours with distant metastases, gemcitabine has become the standard of care after a small randomized trial showed a statistically significant improvement in cancer related symptoms and a modest improvement in overall survival, compared with a regimen that was 5FU based (5.6 versus 4.4 months). The results of this study, and those of several others, have led to the widespread acceptance of gemcitabine as first-line therapy in patients with advanced pancreatic cancer.

The recent elucidation both of the mechanisms involved in pancreatic cancer carcinogenesis and the related molecular events, has led to several distinct therapeutic advances, including many novel target agents, such as monoclonal antibodies against EGFR, EGFR-tyrosine kinase inhibitors, monoclonal antibody against VEGF, farnesyl transferase inhibitors, matrix metalloproteinase inhibitors, COX 2 inhibitors, and the development of gene therapy to target pancreatic cancer.

metalloproteinases inhibitors

A novel family of targets relevant to pancreatic cancer and other malignancies are the metalloproteinases (MMPs). The matrix metalloproteinases are a family of enzymes that are secreted by connective tissue cells, inflammatory phagocytes, and a number of different transformed cells. They are responsible for normal turnover and remodelling of the extracellular matrix and are capable of breaking down most components in the extracellular matrix, including collagen, laminin, fibronectin, elastin, and serpin [2].

These enzymatic activities contribute to the degradation of the basement membrane and the extracellular matrix, thereby contributing to local tumour growth, invasion of blood vessels, and subsequent establishment of metastases. About 18 different MMPs have been identified [3], varying in their substrate requirement and potency; they are produced and secreted in latent forms that require extracellular activation. MMP expression is upregulated in a variety of malignancies and correlates with the invasive and metastatic potential of thyroid, prostate, ovarian, gastric, lung, head and neck and colorectal carcinomas. MMPs seem to play an important role in the pathogenesis of pancreatic cancer that overexpress particularly MMP-2 and MMP-9, implicated in tumour angiogenesis and also MMP-7, and MMP-11 [4].

Synthetic MMP inhibitors (MMPIs) have been developed and are being tested against a variety of tumours. The first orally bio-available MMPI to enter in clinical trials is Marimastat (BB2516); which has been demonstrated to be active against several MMPs [5].

On the basis of the findings of phase I and II trials [6] with marimastat in advanced pancreatic cancer, a large randomized study was conducted comparing gemcitabine (1000 mg/m² weekly) with marimastat at three dose level (5, 10, 25 mg b.i.d.) in first line therapy; gemcitabine showed a superior response rate, median survival and clinical benefit compared with marimastat [7].

The 1-year survival was 19%, the same in the two treatment arm. Marimastat was, generally, well tolerated and seems to have an acceptable toxicity profile at therapeutic doses, with most frequent side-effect being represented by musculo-skeletal toxicity (35% of patients in the 25 mg arm).
Another phase III study compare another MMPI, BAY12-9566, with gemcitabine in the treatment of advanced pancreatic: gemcitabine achieved a superior survival and PFS (Table 1) [8]. At present, the available studies of MMPIs showed that these agents have apparently no role in the treatment of advanced pancreatic cancer. Nevertheless, we should consider mainly these agents have a cytostatic effects making them more appealing for studies in patients with early stage non metastatic pancreatic cancer, or potentially in the adjuvant setting, in view of the its ease of administration and manageable tolerability [9].

### farnesyl transferase inhibitors

Mutation in the ras genes are most commonly found in human malignancies, k-ras is the family member most frequently implicated in solid tumours and pancreatic cancer has the highest frequency (>85%) of k-ras mutation among all human cancer [10].

These mutations are responsible for permanent activation of the k-ras protein that encode a family of proteins important in many of the signal transduction pathways, involved in cell growth, differentiation and apoptosis depending upon the protein modification acted by farnesilation [11]. The inhibition of K-ras gene function through inhibition of farnesyl protein transferase seemed a rational target in pancreatic cancer. Tipifarnib (Zarnestra, R115777) belongs to the class of farnesyltransferase inhibitors, which competitively inhibit the enzyme farnesyl protein transferase, important for modification in Ras protein required for Ras activity.

In phase I and II clinical trials tipifarnib was well tolerated, and the recommended dose was an oral dose of 200 mg/mq b.i.d. The principal toxicities were myelosuppression and fatigue, no pharmacokinetic interaction was documented between tipifarnib and gemcitabine.

In a recent randomized phase III study, which compare gemcitabine with placebo versus gemcitabine with tipifarnib in advanced pancreatic cancer, the combination of gemcitabine and tipifarnib demonstrated an acceptable toxicity profile but it was not able to prolong overall survival and progression free survival, response rate and QoL.

The incidence of hematologic toxicity was higher in the tipifarnib arm, mainly consisting of reversible neutropenia which was reported grade 3 to 4 in 40% of patients. The principal non hematologic toxicities in the tipifarnib arm were diarrhea and hypokalemia [12].

The apparent failure of tipifarnib in advanced pancreatic cancer has a probable explanation with the insufficient inhibition of the protein farnesylation at clinically tolerable concentration, and with the heterogeneity of pancreatic tumors and their dependence on multiple pathways. Given the favorable toxicities of tipifarnib, the role of Kras mutation in pancreatic cancer, additional development of tipifarnib, particularly in earlier stage of pancreatic cancer, seems warranted.

### COX-2 inhibitors

Mounting evidence suggests that COX-2 gene is overexpressed in much premalignant, malignant and metastatic human cancer, including pancreatic cancer. COX-2 protein expression has been demonstrated in 67 to 90% of pancreatic tumors [13, 14].

The COX-2 inhibitors have been show to inhibit the growth and metastasis of established tumors, the probable molecular mechanisms of the antitumor effect of COX-2 inhibitors may be due to inhibition of angiogenesis, but these mechanisms have not fully elucidated. The preliminary report of a phase I study suggested that COX-2 inhibitors celecoxib, may enhance the myelotoxicity of gemcitabine. Nevertheless, in a phase II trial of this combination, no unexpected toxicity was noted, and 17% of patients had a partial response [15, 16].

### EGFR inhibitors

A key molecular event in the development of pancreatic cancer is the overexpression of the epidermal growth factor (EGFR) and activation of its downstream signalling molecules. This receptor is a membrane link with an extracellular domain, a single α-helix transmembrane domain and an intracellular domain with tyrosine-kinase activity; ligand binding leads to homo or heterodimerization with other HER protein and causes activation of tyrosine-kinase and autophosphorylation. This mechanism plays a central role in controlling the activity of the Ras-Raf-MEK-ERK signalling pathway, bringing about a change in proliferation, angiogenesis, apoptosis and ability to give metastases [17].

Two major strategies have been developed to target EGFR: the use of monoclonal (chimeric or humanized) antibodies

**Table 1.** Randomized trials in advanced pancreatic cancer with single-agent gemcitabine vs. MMPI or farnesyltransferase inhibitors

<table>
<thead>
<tr>
<th>First Author</th>
<th>Treatment</th>
<th>No. of patients</th>
<th>Median survival</th>
<th>1-Year survival</th>
<th>Median PFS</th>
<th>Tumor control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bramhall</td>
<td>Gemcitabine</td>
<td>103</td>
<td>5.6</td>
<td>19</td>
<td>3.8</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Marimastat</td>
<td>311</td>
<td>3.6</td>
<td>16</td>
<td>1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Moore</td>
<td>Gemcitabine</td>
<td>139</td>
<td>6.6</td>
<td>25</td>
<td>3.4</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>BAY12-9566</td>
<td>138</td>
<td>3.7</td>
<td>10</td>
<td>1.7</td>
<td>30</td>
</tr>
<tr>
<td>Bramhall</td>
<td>Gemcitabine</td>
<td>119</td>
<td>5.4</td>
<td>17</td>
<td>3.2</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>Gemcitabine+Marimast</td>
<td>120</td>
<td>5.4</td>
<td>18</td>
<td>3.0</td>
<td>61</td>
</tr>
<tr>
<td>Van Custem</td>
<td>Gemcitabine</td>
<td>347</td>
<td>6.0</td>
<td>24</td>
<td>3.6</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Gemcitabine+Zarnestra</td>
<td>341</td>
<td>6.4</td>
<td>27</td>
<td>3.7</td>
<td>59</td>
</tr>
</tbody>
</table>
**Verox inhibitors**

Bevacizumab is another example of human antibody that can be used in advanced pancreatic cancer therapy; a lot of studies evidenced the role of VEGF on angiogenesis, growth and metastasis in pancreatic cancer describing how an up-regulation of VEGF gene is associated with a poor prognosis [29–32].

The combination of bevacizumab and chemotherapy has recently been showed to increase the response rate and extending the progression free survival (estimated survival rate after 1 year was 53% which compares favourably with the historical control of approximately 18%) of patient with advanced pancreatic cancer without influencing the overall survival. The toxicity profile of the combination was acceptable with 36% of patients developing grade 3–4 neutropenia [33–35].

**NF-κB inhibitors**

The Rel/NF-κB family of proteins are inducible dimeric transcription factors that recognize and bind a common sequence motif in nuclear DNA. NF-κB, the major transcription factor in this family, is a p50/RelA heterodimer (p50/p65) present in the cytoplasm of almost all cells. NF-κB regulates cell growth and apoptosis, as well as expression of various cytokines, adhesion molecules, and their receptors. NF-κB is constitutively activated in approximately 67% of pancreatic adenocarcinomas, but not in healthy pancreatic tissue, this factor might contribute to the aggressive growth and drug resistance characteristic of pancreatic cancer [36].

At present however, no NF-κB- specific agents are available for clinical development and the only target for inhibition of NF-κB seems to be proteasome inhibitors that lock NF-κB activity in cells by blocking the degradation of I-κB.

### Table 2. Principal new target therapies

<table>
<thead>
<tr>
<th>Target</th>
<th>Mutation/ expression rate in pancreatic cancer (%)</th>
<th>Novel agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor tyrosine kinase</td>
<td>90</td>
<td>MAb: cetuximab, ABX-EGF, EMD72000</td>
</tr>
<tr>
<td>EGFR</td>
<td></td>
<td>TKI: gefitinib, erlotinib, EKB-569</td>
</tr>
<tr>
<td>HER2/Neu</td>
<td>10</td>
<td>Herceptin, CI-1033</td>
</tr>
<tr>
<td>Ras-Raf-MEK-ERK</td>
<td>90</td>
<td>FTIs: R115777, SCH66336, BMS-214662</td>
</tr>
<tr>
<td>Signaling pathways</td>
<td></td>
<td>17-AAG (non specific)</td>
</tr>
<tr>
<td>Ras</td>
<td></td>
<td>CCJ-779, RAD001</td>
</tr>
<tr>
<td>MEK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P13K/Akt pathways</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Akt mTOR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other molecular targets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COX-2</td>
<td>75</td>
<td>Celecoxib, rofecoxib</td>
</tr>
<tr>
<td>LOX</td>
<td></td>
<td>LY293111</td>
</tr>
<tr>
<td>IL-8</td>
<td>70</td>
<td>ABX-IL8</td>
</tr>
</tbody>
</table>

Directed against the external domain of the receptor and the use of small molecules that compete with ATP for binding to the kinase pocket [17–19].

Immunohistochemistry can detect EGFR expression in more than 90% of pancreatic cancer specimens; this factor enables the selection of patients who really could get benefit from ‘targeted therapy’ with these agents [20, 21]. Starting from this consideration Xiong et al. tested gemcitabine alone versus gemcitabine in association with cetuximab, a chimeric antibody against EGFR, in patients, with locally advanced or metastatic pancreatic cancer, as first line chemotherapy.

Cetuximab was given in a loading dose of 400 mg/mq, followed by weekly doses of 250mg/mq for 7 weeks. Gemcitabine was administrated at 1000 mg/mq for 7 weeks, followed by 1 week of rest. In subsequent cycles, Cetuximab was given weekly and Gemcitabine was administered for 3 weeks every 4 weeks.

After 2 courses of therapy, 5 patients (12%) of 41 achieved a partial response, and 16 (39%) had stable disease. The 1-year survival rate was 32.5% in the Cetuximab plus Gemcitabine arm [22].

Treatment related toxicities were mild and included skin rash, fatigue and fever. This encouraging activity prompted the proposal of a phase III trial comparing gemcitabine versus gemcitabine plus cetuximab by the US Southwest Oncology Group.

Many small molecules tyrosine kinase inhibitors (TKI) of EGFR have been synthesized and are in different phases of clinical development, including gefitinib, erlotinib and EKB-569 [23–25]. The potential advantages of TKI include the potentially easy production of large quantities of drugs and the fact that one molecule can potentially inhibit a family of tyrosine kinase that share a similar structure. At present, TKIs have been studied in combination with gemcitabine only in advanced pancreatic cancer. The results of a phase 1 trial of EKB-569 in combination with gemcitabine for advanced pancreatic cancer were presented at the 2003 ASCO annual meeting; the dose limiting toxicities were grade 3 diarrhea and elevation of transaminase, the maximum tolerated dose (MTD) for the combination was EKB-569 25 mg plus gemcitabine 750mg/mq. 21 patients were treated at the MTD, allowing adequate assessment of the antitumour activity of this combination [26].

Another trial has been presented at ASCO annual meeting 2005; Goldstein et al. evidenced the differences between patients treated with gemcitabine alone versus gemcitabine plus erlotinib, an oral TKI. 569 patients with advanced pancreatic cancer were eligible for the study; in the erlotinib arm, the overall survival, the 1-year survival and the tumor control rates were significantly better than gemcitabine arm [27].

Recently Jimeno et al. have showed that the contemporary use of cetuximab and erlotinib could, also, represent a new strategy in pancreatic cancer therapy; the use of erlotinib seems to amplify gene expression and increase transcription of EGFR receptor, avoiding antiblastic effects; using cetuximab we could interfere with erlotinib induced EGFR up-regulation and obtain anticancer effects [28].
Bortezomib, a boronic acid dipeptide, is a unique and specific inhibitor of the proteasome pathway [37], that inhibits the proteasome pathway rapidly and in a reversible manner by binding directly with the 20S proteasome complex and blocking its enzymatic activity. This therapy has just been experimented in a lot of phase I and II clinical trials for advanced hematologic disease; the most common (occurring with a frequency of at least 20%) toxicities were gastrointestinal side effects, transient thrombocytopenia, fatigue, fever, and peripheral neuropathy [38, 39]. Most of these toxicities were of grade 1 to 2 severity. Less common side effects included rash (15%), headache (20%), and dizziness (10%) [39].

Crossover drug resistance against conventional treatment and develop synergetic action with Gemcitabine or Paclitaxel represent, at the state of the art, the rationale for Bortezomib use in pancreatic cancer therapy. At present, only a few phase I clinical trials are open and more studies are needed to corroborate its rule in solid cancer treatment [40–42].

disclosures

Dr. Casciu has indicated that he is currently conducting research sponsored by Merck and that he is a member of the Merck speakers’ bureau.

references