Update on Targeted Therapies in Pancreatic Cancer

Pancreatic cancer is remarkable for relatively late presentation due to vague symptoms and deeper anatomical location of pancreas which renders it non palpable on clinical examination. Though cure is possible in early stage pancreatic cancer, locally advanced or metastatic disease remains largely incurable despite of advances in surgical techniques, radiotherapy and chemotherapy. At present, only 20% of patients present with early-stage operable tumors [1]. Aggressive tumor biology and limited efficacy of conventional therapies lead to rapid progression of disease and higher cancer specific mortality. One year survival of these patients is 23% and 5 years survival is dismal 5% [2]. Approximately a quarter of a million people world-wide die annually from this disease [3]. This point towards a desperate need to look for a therapy which can better the existing response rates of chemotherapy and improve outcome.

Extensive research in the field of cancer genetics in last few decades has brought a paradigm shift in understanding of cancer biology. The understanding has shifted from cancer being a conglomeration of abnormal cells to a disease resulting from accumulation of multiple genetic and cellular changes and their ultimate culmination as a neoplasm with peculiar molecular characteristics. The understanding of molecular milieu and various pathways involved in cancer proliferation and propagation gave an appealing concept of targeting these mechanisms for the control of cancer. Since targeted therapies are against a specific molecular motif in cancer cells, they are supposed to spare normal host cells relatively [cf conventional chemotherapy, which can not differentiate amongst cancer cells and normal cells]. Remarkable results of Imatinib in Chronic Myeloid Leukemia and use of Anti CD20 antibody Rituximab in B cell lymphoid neoplasm, herald the era of exciting target therapies.

Various molecular targets have been identified in pancreatic cancer. Many phase I and II studies have shown significant improvement in outcome with these agents. However in phase III randomized studies, only few agents could live up to the promise. Molecular targeted therapies have been used as an adjunct to chemotherapy,
as a radio sensitizer and as single agent as well. Table 1 shows some of the molecular targets more frequently observed in pancreatic cancer.

**VEGF ANTAGONIST: Targeting Angiogenesis**

Angiogenesis is a biological process essential for normal functioning of body. When the same process is adopted by tumors, it becomes a major hindrance in containing a tumor at place of origin and treating it. Vascular endothelial growth factor (VEGF) is the most important ligand of angiogenesis pathway. The VEGF growth factor family consists of five members (VEGF- A to E) that bind to and activate three distinct receptors. VEGF-A binds to VEGFR1 and VEGFR2, and placental growth factor and VEGF-B bind only to VEGFR1. VEGF-C and VEGF-D bind to VEGFR2 and VEGFR3 [5]. Biological significance of VEGFR1 is less well elucidated but, VEGFR2 plays important role in down-stream signaling. Three major downstream pathways involved in angiogenesis are PI3K pathway, MAP Kinase pathway and Src kinase pathway. Additionally, VEGF is a regulator of vascular permeability, vasodilatation, endothelial migration and gene expression. Studies have demonstrated a correlation between tumor VEGF-A levels, blood vessel density, tumor size and local metastasis of pancreatic adenocarcinoma [6,7].

Bevacizumab is a humanized monoclonal antibody targeted against all isoforms of VEGF-A. In animal models it was found to be active against pancreatic cancer cell lines and was found to be effective both as a single agent and in combination with chemotherapy in early trials. [8,9]. Besides direct antitumor activity, potential of Bevacizumab as a radiosensitizer was also realized. In a phase I study, Bevacizumab was administered with capecitabine and radiotherapy to 47 patients of locally advanced pancreatic cancer. This study showed that Bevacizumab can safely be combined with chemoradiation, and 20% patients had partial response [10]. A phase II study involving 52 patients assessed the efficacy of Bevacizumab with Gemcitabine as a first-line treatment for metastatic pancreatic cancer. Gemcitabine was administered at 1000 mg/m 2 weekly for 3 out of 4 weeks and Bevacizumab at 10 mg/m 2 on days 1 and 15 in a 28-days treatment cycle. This combination 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 [11].

A phase III trial involving 602 patients receiving either Gemcitabine with placebo or Bevacizumab is undergoing and interim results show that median failure-free survival slightly favored the addition of Bevacizumab (4.8 versus 4.3 months); overall survival was not improved at 5.2 months relative to 5.8 months in the patients who received Gemcitabine together with placebo. Final results of this study will elaborate more on effect of Bevacizumab in metastatic pancreatic cancer [12].

**Inhibition of EGFR pathway**

The Epidermal Growth Factor Receptor (EGFR) is a 170 kD transmembrane protein, a member of the HER family of transmembrane receptor tyrosine kinases which comprises four members: ErbB1/HER1/EGFR, ErbB2/HER2/neu, ErbB3/HER3 and ErbB4/HER4. Structurally, HER receptors consist of an extracellular ligand-binding domain, a transmembrane domain and an intracellular protein kinase domain. Both extracellular ligand binding domain and intracellular kinase domain are targeted to achieve EGFR blockage. Over expression of EGFR is found in 69% of pancreatic cancers [13]. EGFR signaling is associated with tumor proliferation, invasion and metastasis and is associated with adverse outcome.

Cetuximab, trastuzumab, erlotinib, lapatinib and gefitinib are some of the currently available EGFR inhibitors which have been tried in pancreatic cancer. Cetuximab [a chimaric monoclonal antibody against EGHFR] and trastuzumab [a humanized monoclonal antibody against EGHFR] binds to extracellular domain to receptor and act by competitive inhibition of ligand binding.

**Cetuximab:** in a phase II trial to evaluate the efficacy of Cetuximab in combination with gemcitabine in locally advanced and recurrent pancreatic cancers, patients received weekly Gemcitabine [1000 mg/m2] 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 250 mg/m2 weekly infusions. The median survival was 7.1 months. Treatment was generally well tolerated and resulted in a 1-year survival of 32.5%, compared with
18% for Gemcitabine alone in historical series. Suggesting that the combination of Gemcitabine and Cetuximab may be more efficacious than Gemcitabine monotherapy [14]. However, a phase III trial to study Cetuximab alone or in combination with Gemcitabine as first-line treatment for advanced pancreatic cancer disappointingly failed to show any significant benefit of Cetuximab addition [15]. At present role of Cetuximab in pancreatic cancer remains investigational.

**Matuzumab:** Matuzumab is an experimental biological agent in its developmental stage. It is a humanized monoclonal antibody targeting EGFR. A phase I trial used 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 dose of 800 mg matuzumab per week. The median survival for patients was 3.7 months and stable disease was observed in 65% of patients. (Range: 0.4 – 12.2 months) [16]. Further Phase II and III studies are required to elucidate role of this agent in pancreatic cancer.

**Trastuzumab:** Role of Trastuzumab in patients of breast cancer over-expressing HER2neu is well established. Her2 is over expressed in 50% patients of pancreatic cancer [17]. The targeting of Her-2 has been studied in advanced pancreatic cancer. A Phase II trial of the Gemcitabine/trastuzumab combination enrolled 34 patients of Her-2 expressing pancreatic cancer [18]. The objective response rate to the combination was 6%, and was not superior to Gemcitabine alone.

**Small Molecule EGFR receptor inhibitors:** Small molecules compete with ATP for ATP binding site on intracellular tyrosine kinase domain of EGFR. As a result they prevent receptor phosphorylation and further downstream signaling. Erlotinib and Gefitinib are two main agents of this group.

In a recent landmark phase III trial of advanced pancreatic cancer, 569 patients were randomized to receive Gemcitabine (1000 mg/m2/week for 7 out of 8 weeks) with placebo or 100 mg Erlotinib daily. Treatment arms were compared using log-rank tests stratified by performance status, extent of disease, and pain score at baseline. The 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%). Moreover, there was a significant reduction in the hazard of death (hazard ratio 0.82 [95% CI 0.67 – 0.97]). The HR of 0.82 represents an overall 22% improvement in survival. There was a higher incidence of some adverse events with erlotinib plus gemcitabine, but most were grade 1 or 2. The appearance of the characteristic rash was found to be associated with better survival [19]. This trial provides proof of principle of targeting HER1/EGFR in pancreatic cancer and shows erlotinib can improve survival when used concurrently with chemotherapy. Erlotinib became the first targeted therapy approved by the FDA for use in pancreatic cancer in November 2005 and recommended dose is 100mg per day. In another trial, role of Erlotinib in Gemcitabine refractory advanced pancreatic cancer was investigated. [20] Patients were administered 1000 mg/m2 capecitabine twice daily for 14 out of 21 days, and 150 mg Erlotinib daily. The regimen was well-tolerated with rash and diarrhea being the most frequently observed toxicities. Overall response rate was 10% and median survival was 6.5 months. Considering the dismal outcome of Gemcitabine refractory pancreatic cancers, these results are encouraging enough to be explored further in larger and randomized trials

Radio-sensitizing property of Erlotinib is also recognized. In a phase 1 trial from Brown university combination of weekly Gemcitabine, Pacalitaxel and daily Erlotinib with radiotherapy [50.4 GY] to primary tumor was tested. Maximum tolerated dose of Erlotinib was 50mg/m2 and median survival was 14 months. 46% patients had PR. However, toxicity of concurrent therapy was more [21]. Further studies are required for assessment of role of Erlotinib in concurrent chemo-radiotherapy.

Gefitinib: Data on the role of gefitinib in pancreatic cancer is not as robust as that of erlotinib. One phase II study of gefitinib and Gemcitabine in patients with inoperable or metastatic pancreatic cancer has shown results similar to those of Erlotinib with Gemcitabine [22]. Result of Gefitinib in upfront concurrent chemo-radiotherapy or in combination with docetaxel in gemcitabine refractory cases were not encouraging [23, 24].
Lapatinib: Lapatinib is a potent inhibitor of EGFR and Her2 and this agent can offer benefit of double pathway blockage in pancreatic cancer. A phase I clinical trial tested Lapatinib in combination with Gemcitabine or the Gemcitabine/Oxaliplatin doublet in patients of advanced untreated pancreaticobiliary cancer [25]. The study was notable for a 23% objective response rate in evaluable patients. However no conclusive benefit of Lapatinib was found. Small number of patients and inclusion of other biliary malignancies might have impact on results.

Table 2 summarizes the results of various EGFR inhibitors.

**Targeting Telomerase**

Telomeres are non-coding sequence (TTAGGG) at the ends of chromosomes. Their main role is to prevent attrition of genetic material from chromosome due to repeated multiplication. With every cell division telomere length shortens and after a critical shortening, cell is rendered incapable of further multiplication. This stage is called “replicative senescence”. After certain number of cell doubling, shortened telomere also lead to apoptosis of aged cells. In a way telomere guard against loss of genetic material from ends of chromosomes and also help in apoptosis of cells which have completed their desired replication.

Telomerase is the enzyme which synthesizes telomeres at the ends of chromosomes. In normal cells telomerase are in inactive state; hence cells undergo senescence after desired number of replications. While in many cancer cells, detectable telomerase activity is identified; which replenish the shortened telomere after every replication and impart immortality to these cancer cells. Furthermore, the inhibition of telomerase in tumor cells leads to rapid telomere shortening and apoptosis [26]. An in vitro study identified that telomerase related gene are up-regulated in pancreatic cancer cells but not in their benign counter parts [27]. It was hypothesized that if telomerase activity can be tamed then immortality of a cancer cell can also be controlled.

The hTERT, or "human Telomerase Reverse Transcriptase," is a ribonucleoprotein and is the catalytic subunit of the enzyme telomerase. It is observed that hTERT subunit is degraded into small peptides by tumor cells and presented on cell
surface in conjunction with MHC molecules. This antigen presentation leads to activation of cytotoxic T cells against peptide bearing cancer cells. This strategy leads to selective elimination of telomerase expressing cancer cells [28]. A telomerase-derived peptide GV1001 has shown promising results in a dose escalation phase I/II study in patients of non-resectable pancreatic cancer [29]. Measurable immune response was observed in 75% of the patients on intermediate dose of peptide. 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 of peptide. After 300 days, all surviving patients were those who had demonstrated an immune response to the peptide. These initial results are quite encouraging and phase III trial is underway to investigate possible role of GV1001 in current management of advanced pancreatic cancer.

**Inhibition of RAS/RAF signaling**

Four oncogenic RAS proteins have been described so far; HRAS, NRAS, KRAS-4A and KRAS-4B. They exert their biological functions by assembling signaling complexes at the cell membrane that activate intracellular signaling cascades and changes in various intracellular processes affecting cell survival, proliferation, differentiation, and senescence [30]. Unregulated RAS signaling leads to uncontrolled proliferation, migration, invasion and resistance to apoptosis [31].

KRAS mutations are exceptionally frequent events in pancreatic cancers and predominantly occur at codon 12 [32]. Inhibition of RAS activity is thus an appealing anti-cancer strategy. Farnesylation of RAS protein is a critical step in its functioning. Farnesyl transferase inhibitors (FTI) have been used with some success in acute leukemias. However a FTI-Tipifarnib was found to be ineffective when tested in patients of pancreatic cancer [33]. Newer antisense technology is being investigated as a mode of RAS inhibition. A compound ISIS-2503 is an antisense nucleotide against HRAS. In phase II trial of locally advanced and metastatic pancreatic cancer cases, combination of Gemcitabine and ISIS-2503 produced overall response rate of 10.4% and median
survival of 6.6 months. The combination was well tolerated as well. May be in future, better understanding of antisense agents can open a new therapeutic window for advanced pancreatic cancers.

The small molecule multikinase inhibitor Sorafinib inhibits RAF, MEK, VEGFR-2, VEGFR-3 and platelet-derived growth factor receptor-beta. In a phase I trial the combination of 400 mg Sorafinib twice daily with 1000 mg/m² Gemcitabine was found to be relatively well-tolerated, and 13(56.5%) pancreatic cancer patients showed evidence of disease stabilization [34]. Further research is required to explore potential role of Sorafinib in pancreatic cancer.

**Gastrin inhibition**

Gastrin is a local peptide hormone facilitating secretion of digestive enzymes. Gastrin receptors are expressed on pancreatic cancer cells and gastrin was found to stimulate proliferation of pancreatic cancer cells [35]. Gastrin receptor antagonist Gastrazole was investigated in two trials of advanced pancreatic cancer. When compared with placebo, Gastrozole produced significantly better median survival and 1 year survival. However when it was compared with 5FU, no significant difference in outcome was noted [36].

**COX2 inhibition.**

Cyclooxygenases (COX) are enzymes involved in Prostaglandin synthesis and are primarily involved as mediator of inflammation. COX2 was found to be over expressed in pancreatic cancer cells and COX2 inhibitors have shown antiproliferative effects on pancreatic cancer cell lines. [37]. In various phase I studies COX2 inhibitor celecoxib has shown mixed results. Combination of celecoxib with Gemcitabine with radiation showed increased efficacy [38] but no benefit was noted when it was combined with 5FU [39]. A 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% [40].
Summary

Despite success in various cancers and promising data in preclinical studies in pancreatic cancer; most targeted therapy failed to bring remarkable improvement in outcome of these patients. While there is cumulative rise in knowledge of cellular and molecular biology of pancreatic cancer, the same could not be translated into clinical benefit to the patients. Why is it so that when clearly identified targets and their inhibitors are available- desired clinical results elude researchers? Probable answer lies in potential mechanisms of acquiring resistance by cancer cells against these agents. Following blockade of one signal transduction pathway a cancer cell may overexpress an alternative pathway and in this way it may minimize effect of targeted agent. Upregulation of vascular endothelial growth factor (VEGF) has been described in cell lines with acquired resistance to Cetuximab and other EGFR-directed antibodies [41] . Envisaging this hypothesis, use of combination of targeted agent against different pathways looks an appealing concept. However some initial results of these combinations also have been far from impressive. Novel chemotherapy and biologic therapies should constantly be explored given the poor results achieved with standard therapy and limited success with currently available targeted therapies.

References


Table 1: Molecular targets for pancreatic cancer therapy*.

<table>
<thead>
<tr>
<th>Molecular target</th>
<th>Frequency of Mutation/expression (%)</th>
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<tbody>
<tr>
<td>VEGF</td>
<td>93</td>
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<tr>
<td>KRAS</td>
<td>95</td>
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<tr>
<td>EGFR [HER1]</td>
<td>69</td>
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<tr>
<td>ErbB2</td>
<td>33-52</td>
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<td>Gastrin</td>
<td>23-91*</td>
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<tr>
<td>COX2</td>
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<td>Activated AKT/mTOR</td>
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<td>Sonic hedgehog</td>
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<tr>
<td>IGF1R</td>
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*Adapted from Danovi SA, Wong HH, Lemoine HR. Targeted therapies for pancreatic cancer. British Medical Bulletin 2008; 87: 97–130, with modification
Table 2 Results of studies involving various EGFR inhibitors.

<table>
<thead>
<tr>
<th>HER inhibitor</th>
<th>Study</th>
<th>Number of patients</th>
<th>Response rate</th>
<th>Overall survival</th>
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<tbody>
<tr>
<td>Cetuximab</td>
<td>Xiong et al (14)</td>
<td>40</td>
<td>12.5%</td>
<td>7.1 months</td>
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<td>Trastuzumab</td>
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<td>6%</td>
<td>7 months</td>
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<td>Erlotinib</td>
<td>Moore et al (19)</td>
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<tr>
<td>Lapatinib</td>
<td>Safran et al (25)</td>
<td>25</td>
<td>23%</td>
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