London Pancreas Workshop 2008

Date 2nd May 2008

Time 1000-1730

Venue: Charterhouse Square, Barts and the London, London

Final Programme

Diagnostics Peter Fairclough and Ajit Abraham			
1000-1020	Role of pancreatic fluid and serum in diagnosis of pancreatic tumours	Mike Goggins	Johns Hopkins, USA
1020-1040	Urine biomarkers	Tatjana Crnogorac-Jurcevic	Barts and the London
1040-1100	Role of PET and CT/PET scan in HPB tumours	Norbert Avril	Barts and the London
Tea			
Therapeutics David Propper and Satya Bhattacharya			
1130-1150	Extensive surgery: is there a role?	Hemant Kocher	Barts and the London
1150-1210	Capri trial: what can we learn?	Angela Marten	Heidelberg
1210-1230	Cetuximab trial	Robert Krempien	Heidelberg
Lunch			
Laboratory Science Richard Grose and Helen Hurst			
1400-1430	Methylation in pancreatic cancer	Mike Goggins	Johns Hopkins, USA
1430-1530	Mouse models in pancreatic cancer	David Tuveson	Cambridge
Tea			
New Translational Trials/models Nick Lemoine and Ian Hart			
1600-1630	Pancreatic cancer stem cells: do they exist	Malcolm Alison	Barts and the London
1630-1710	Viral gene therapy	Iain McNeish	Barts and the London
1710-1730	Photodynamic therapy in HPB tumours	Steve Pereira	University College Hospital

Role of pancreatic fluid and serum in diagnosis of pancreatic neoplasia

Mike Goggins, Johns Hopkins University, Baltimore, USA.

Pancreatic juice fluids are being investigated as a source of markers of pancreatic neoplasia. Pancreatic juice is collected during routine upper GI endoscopy after secretin infusion, making it a potentially optimal specimen from which to analyze markers in patients who have diffuse abnormalities of the pancreas by imaging but no discrete mass or as a means to examine the pancreatic ductal system as part of screening protocols to detect microscopic neoplasia in high-risk individuals. Fine needle aspirates (FNAs) are best used to sample focal lesions identified by imaging. Cytologic interpretation of FNAs of pancreatic lesions is often inconclusive. Thus, molecular markers hold the potential to improve the diagnostic yield of FNAs. Molecular markers of pancreatic neoplasia including mutations and aberrant methylation can be detected in pancreatic fluid. Prospective studies are underway to evaluate the diagnostic utility of identifying and quantifying pancreatic juice molecular markers.

Urine biomarkers

Tatjana Crnogorac-Jurcevic, Institute of Cancer, London

Urine has recently been explored as a potential source of biomarkers, as it is an easily and non-invasively obtainable bio-fluid. In comparison with plasma, urine proteins are less complex and more thermostable, and most common proteins (albumin, uromodulin) comprise a lesser proportion of the urinary proteome, so sample processing requires less pre-cleaning/fractionation. As approximately 50% of urinary proteins are soluble products of glomerular filtration of plasma, a substantial number of proteins in urine arise from extrarenal sources.

In addition to urological cancers, cancer-related proteins have been identified in the urine of patients with lung, ovarian and breast cancers. This has prompted us to search for the signatures of pancreatic adenocarcinoma in urine specimens.

We performed 2-D DIGE analysis of urine samples collected from patients with pancreatic cancer and chronic pancreatitis and compared them to healthy urines. A total of 127 statistically valid ($P<0.05$), differentially expressed protein spots were detected, 101 of which were identified using MALDI-TOF MS. Several of these have previously been associated with pancreatic cancer, proving that urine is a valuable source of non-invasive diagnostic markers for pancreatic diseases.

PET and PET/CT in HBP tumours

Norbert Avril, Institute of Cancer, London

Positron emission tomography (PET) is a functional imaging technology which enables the visualization, characterization and quantification of biological processes *in vivo*. By using positron emitting radiotracers, PET provides unique information about the molecular and metabolic changes associated with disease (molecular imaging). Glucose metabolism is often increased in malignant tumours resulting in increased cellular uptake of the glucose analogue F-18 fluorodeoxyglucose (FDG). Imaging the metabolic activity of tumours provides sensitive and specific information about the extent of disease. Combined positron emission tomography and computed tomography (PET/CT) represents the latest technology, which acquires PET and CT images that are concurrent and co-registered, merging the functional information from PET with the anatomical information from CT.

In pancreatic cancer, FDG-PET is useful in selected patients in whom conventional imaging findings are inconclusive. FDG-PET is particularly helpful in the diagnostic work-up of patients with suspected pancreatic lesions. A recent meta-analysis compared PET/CT versus CT to distinguish benign from malignant lesions. Sensitivity and specificity of FDG-PET ranged from 71-100% and 53 to 100%, respectively. On the other hand, sensitivity and specificity of CT alone ranged from 53-100% and 0-100%, respectively.

In hepatocellular carcinoma (HCC), FDG-PET is primarily useful in assessing the degree of differentiation and in staging moderately and poorly differentiated tumours. Sensitivity of FDG-PET for the detection of HCC is ranging between 50-70%. The limited sensitivity is due to the low level of FDG uptake in well-differentiated tumours; however, FDG-PET may be superior to CT in detecting extrahepatic spread.

Cholangiocarcinomas and gallbladder cancers are generally FDG-avid. FDG-PET/CT and contrast enhanced CT provide a comparable accuracy for primary intra- and extra-hepatic cholangiocarcinomas, although FDG-PET/CT is superior in detecting distant metastases. Both modalities have limited use in the evaluation of local lymph node metastases. FDG-PET can be false-positive in inflammatory processes and should be interpreted with caution in patients with primary sclerosing cholangitis and stents in place. PET/CT has been shown to be valuable in detecting distant metastasis.

FDG-PET can also be useful for assessment and early prediction of treatment response. Changes in tumour glucose metabolism precede changes in tumour size and reflect drug effects at a cellular level. FDG-PET allows the prediction of therapy response early in the course of therapy as well as determining the viability of residual masses after completion of treatment. A number of studies have shown that the degree of changes in FDG uptake after initiation of chemotherapy correlates with histopathologic response after completion of chemotherapy.

Extensive Surgery: Is there a role?

Hemant Kocher, Institute of Cancer, London

Aim: To compare outcomes between pancreaticoduodenectomy (PD) and extended pancreaticoduodenectomy (EPD) as well as pylorus preserving pancreaticoduodenectomy (PPPD) from all published comparative studies in the literature.
Methods: Using meta-analytical techniques the present study compared operative details, post-operative adverse events and survival following PD and EPD as well as PD and PPPD. Comparative studies published between 1988 and 2005 of PD versus EPD were included. End points were classified into peri-operative details, post-operative complications including 30 day mortality, and survival as measured during follow up. A random effect model was employed.
Results and Conclusions: Sixteen comparative studies comprising 1909 patients (865 PD and 1044 EPD), including 3 randomized controlled trials with 454 patients (226 PD and 228 EPD) were identified. EPD is associated with a greater nodal harvest and fewer positive resection margins than PD. However, the risk of delayed gastric emptying is increased and no significant survival benefit has been shown. Better designed, adequately powered studies are required to settle this question. 32 studies comprising 2822 patients (1335 PD and 1487 PPPD), including 5 randomized controlled trials with 421 patients (215 PD and 206 PPPD) were included. Both PD and PPPD had similar peri-operative adverse events, however, in overall analysis PPPD has lower mortality and improved long-term patient survival, although this was not reflected in the sub-group analysis.

1: Iqbal N, Lovegrove RE, Tilney HS, Abraham AT, Bhattacharya S, Tekkis PP, Kocher HM. A comparison of pancreaticoduodenectomy with extended pancreaticoduodenectomy: A meta-analysis of 1909 patients. Eur J Surg Oncol. 2008 Mar 18; PMID: 18356005
2: Iqbal N, Lovegrove RE, Tilney HS, Abraham AT, Bhattacharya S, Tekkis PP, Kocher HM. A comparison of pancreaticoduodenectomy with pylorus preserving pancreaticoduodenectomy: A meta-analysis of 2822 patients. Eur J Surg Oncol. 2008 Feb 1; PMID: 18242943

Capri trial: what can we learn?

Angela Märten, University of Heidelberg, Germany

Data from a phase II trial combining chemoradiotherapy with IFN- (CapRI scheme) for adjuvant treatment of pancreatic carcinoma are very encouraging. Therefore, a phase III trial comparing chemotherapy with the CapRI scheme has been initiated in August 2004.
An immunomonitoring focussing on NK cell activity, immunophenotyping of peripheral blood, analysis of tumour-specific cytokine release, tumour lymphocyte infiltration, and analysis of various serum markers accompanied the CapRI trial.
Strong immune reactions caused by IFN- were observed. One day after the first IFN- injection, a strong increase in monocytes, peripheral DC, CD40 expression, CM, and NK cell-mediated cytotoxicity was observed. Four days later, the serum level of TNF- and IL-12 peaked. Later on, a second peak of peripheral DC, an increase of effector memory T cells (EM) in parallel to the multimodality treatment phase and most interestingly antigen-specific T cells appeared. These observations could be nicely explained by a series of events starting with the immune system tackling with the tumor, forced and got on the way by the injection of IFN- and resulting finally in a switch from the innate to the specific immune system.
To enable a screening for predictive markers prior to the end of the trial two groups were formed: the population matching one of the criteria was divided into the groups: a) free of disease more than two years (responder) and b) recurrence within two years (non-responder).
Preliminary data indicate a correlation between NK cell activity and clinical outcome. Responder had responded to the first IFN- challenge with a NK cell mediated cytotoxicity of >30% lysis of K562 cells. Furthermore, responder had significantly more IFN-gamma secreting cells upon IFN- stimulation than non-responder. This was shown in ELISpots using MUC-1 and CA 19.9 as antigens.
Beside this functional parameter the following correlations were observed:
a) Immunophenotyping: Responder had a significant higher increase in CD8+ cells, activated NK cells, peripheral dendritic cells upon INF- stimulation and a decrease in CD25 expression

b) Cytokine serum levels: Responder had a significant higher increase in IL-12 upon INF- stimulation.

c) Plasma markers: Responder showed a trend towards lower sMICA levels compared to non-responder. Most interestingly, subjects showed an increase in siC3b plasma levels up to six months prior to radiological defined recurrence (Figure 5).

d) mRNA: Responder had a significant increase in IP10 in leukocytes compared to no increase in non-respondern.

Final results of a Phase II trial [PARC-Study ISRCTN56652283] for patients with primary inoperable locally advanced pancreatic cancer combining intensity modulated radiotherapy (IMRT) with Cetuximab and Gemcitabine.

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Background: To evaluate the feasibility and efficacy of trimodal therapy using cetuximab in combination with IMRT and gemcitabine in locally advanced pancreatic cancer.

Methods: In this randomized Phase II trial (planned sample size 66 pts), chemotherapy-naive pts. with primary inoperable locally advanced pancreatic cancer were eligible. For both arms initial treatment consisted of radiotherapy, gemcitabine weekly (300mg/m^2), and cetuximab weekly (loading dose 400mg/m^2 day 1, concomitant with RT 250mg/m^2). After trimodal therapy pts in arm A received gemcitabine weekly (1000mg/m^2) over 4 weeks, and pts in study arm B received gemcitabine weekly (1000mg/m^2) over 4 weeks and cetuximab (250mg/m^2) weekly over 12 weeks. IMRT was delivered using an integrated boost concept (54 Gy GTV, 45 Gy CTV) over 5 weeks. Response evaluation (CT) followed at week 12 and every 3 months. All pts were treated with gemcitabine weekly (1000 mg/m^2) over 3 months after end of study. Primary study end point was response; secondary end points were overall survival, secondary resection rate and adverse events.

Results: Between 2/05 and 4/07 68 pts were enrolled (characteristics: pancreatic adenocarcinoma c2 T4 N1 68/68, median age = 62 (range 48-79); M/F = 38/30; ECOG PS 0/1/2 = 17/45/6). Complete response in 1/68, partial response in 23/68, no change in 41/68 and progressive disease in 3/68 pts was found according the RECIST criteria in the CT controls. Median follow-up at present is 11 months, 1-and 2-year survival is 61% and 20 %, respectively, median survival is 15 months. No statistical significance was reached

concerning overall survival between the treatment arms. 40/68 pts were amenable for secondary potentially curative resection. 14 pts could be resected. Overall toxicity was acceptable and consistent with the profiles of the individual agents.

Conclusions: Trimodal therapy for primary inoperable locally advanced inoperable pancreatic carcinoma is safe and resulted in excellent local control and overall survival. No statistical significant difference between the two treatment arms could be found. Distant metastases were the main cause of failure.

DNA Methylation alterations in pancreatic neoplasia

Mike Goggins, Johns Hopkins University, Baltimore, USA.

Pancreatic cancer is an epigenetic disease, as it is a genetic disease, characterized by widespread and profound alterations in DNA methylation. The introduction of genome-wide screening techniques has accelerated the discovery of a growing list of genes with abnormal methylation patterns in pancreatic cancer, and some of these epigenetic events play a role in the neoplastic process. We will discuss recent research findings in pancreatic cancer epigenetics and their biological and clinical implications.

Mouse models of Pancreatic Cancer

Kris Frese and David Tuveson

Li Ka Shing Cambridge Research Institute/CRUK, University of Cambridge, UK

Pancreatic ductal adenocarcinoma (PDA) is a common and lethal cancer, responsible for approximately 200,000 cases and deaths annually world-wide. Our inability to intervene meaningfully has been attributed to the poor activity of systemic therapies and to the late stage of disease presentation. We have generated several genetically engineered murine models (GEMM) of PDA to explore the disease etiology and investigate preclinical applications. These GEMMs harbour pancreatic-specific orthologous mutations in oncogenes (KRAS) and tumour suppressor genes (TrP53, p16Ink4a, SMAD4) that are the canonical genetic alterations found in human pancreatic cancer. Importantly, these GEMMs recapitulate the molecular and pathophysiological features of human pancreatic cancer, including the presence of signature biochemical alterations and chromosomal instability, and the development of widespread metastases and cachexia in effected mice. Using such models, we have explored the cellular origins of pancreatic cancer, identified new pathways involved in the genesis of pancreatic cancer, and investigated the therapeutic response of such models to standard treatments. Collectively, our results should illuminate some features of this malignancy and stimulate new approaches to pancreatic cancer patients.

Pancreatic stem cells: do they exist?

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Two papers appeared to signal that it's time to stop the search for an indigenous stem cell compartment in the mammalian pancreas, concluding that there are no stem or progenitor cells that give rise to families of descendants. Teta *et al.* (Developmental Cell) adopted a simple labelling approach to study developmental and regenerative growth in the mouse pancreas. Sequentially administering two different DNA labels, whereby each label could be separately identified within any cell that had incorporated them, it was reasoned that a DNA-synthesising cell (having taken up the first label which would mark both its daughter cells), would yield double-labelled cells if either of its daughters entered the cell cycle again during the second labelling period. Double labelled cells were very rare, even after massive pancreatic damage, leading to the conclusion that we cannot identify family lines (a cell hierarchy) descended from stem/progenitor cells. Brennand *et al.* (PLoS Biology) also looked at early postnatal growth in the mouse, labelling cells with a histone protein tagged to a fluorescent marker that was stably incorporated into a large, but random number of -cells. The average fluorescence intensity of each labelled cell could be measured and, during further growth, if only certain cells (stem cells) divided and produced progeny that also divided (a hierarchy), then they would produce cells that were 'fluorescent-dull'. In fact the label was equally diluted across the -cell population, suggesting neither a sub-population of stem cells whose subsequent successive divisions would generate particularly lightly labelled cells existed, nor indeed was there any remaining heavily labelled cells (so-called label retaining cells) that might indicate a slowly cycling stem cell population.

So should we call off the search for stem cells in the pancreas? To my mind the critical experiment has not been done. In the liver, an organ in many ways very similar to the pancreas, stem cells from the bile ducts only become activated after injury if hepatocytes are inhibited. The pancreatic ducts have often been proposed to be the location of the elusive stem cell for -cell replacement, so if we could exclusively block only -cell regeneration in the damaged pancreas, a ductal or indeed other intrapancreatic stem cell may yet show its face! Careful inspection of human pancreatic ducts from diabetics reveals buds of insulin-positive cells, and searching for cells deficient in mtDNA-encoded

cytochrome c oxidase reveals patches of cells that are clonally derived, at least in the exocrine pancreas.

Gene therapy for pancreatic cancer

Iain A. McNeish, Institute of Cancer, London.

Given its poor prognosis, it is little surprising that pancreatic cancer has been the subject of much research in gene therapy. The strategies initially employed included genetic prodrug activation therapy (selective expression of a prodrug-activating enzyme within tumour cells) and restoration of tumour suppressor function (e.g p53 and p16). These strategies had some success *in vitro* and in xenograft models, but did not develop into clinical trials.

More recently, promising data have emerged from work using oncolytic viruses. These viruses multiply selectively within cancers, causing cell death, with released mature viral particles infecting neighbouring cells. The tumour suppressor and cell defence mechanisms that viruses subvert are the same as those lost in carcinogenesis. Some viruses are intrinsically tumour-selective in their replication, whilst the large DNA viruses require deletion of key viral genes to achieve selectivity.

The first formal clinical trials tested the E1B-55K deleted adenovirus *dl*1520 (Onyx-015), which was hypothesised to replicate selectively in p53-negative cells. At least 15 clinical trials using *dl*1520 have been completed and a derivative, H101, is licensed in China. Doses of up to 10^{13} particles were safely administered by a variety of routes, including intravenous. In pancreas cancer, single agent activity was poor, but there were demonstrable clinical responses when patients were co-treated with gemcitabine. The second generation adenoviral mutants *dl*922-947 and Δ24 both contain a 24 bp deletion in the E1A CR2 region, which normally binds to pRb. These viruses replicate selectively in malignant cells as they have a deregulated Rb pathway and hence G1/S checkpoint. We have data showing activity of *dl*922-947 in pancreatic cancer models.

Other potential oncolytic viruses for pancreas cancer include reovirus, which was originally thought to replicate in cells with activating Ras mutations, and Herpes simplex virus.

Finally, as progress is made in unravelling the complex genetics of pancreatic cancer, novel targets are emerging, which will form the basis of the next generation of genetic therapies. The difficulties of translating this new knowledge into potential treatments will be discussed.

Photodynamic therapy in HPB tumours.

Steve Pereira, University College London.

The prognosis of patients with pancreatic and biliary tract cancer (BTC) treated with conventional therapies such as stent insertion or chemotherapy is often poor, and new approaches are needed. Surgery is the only curative treatment but is appropriate in less than 20% of cases, and even then is associated with a 5-year survival of less than 30% in selected series. Photodynamic therapy represents a novel treatment for pancreaticobiliary malignancy. It is a way of producing localised tissue necrosis with light, most conveniently from a low-power, red laser, after prior administration of a photosensitising agent, thereby initiating a non-thermal cytotoxic effect, vascular injury and tissue necrosis. As the biological effect is photochemical, not thermal, there is little damage to connective tissues such as collagen and elastin, which helps to maintain the mechanical integrity of hollow organs like the gastrointestinal tract . Although most applications of PDT in gastroenterology to date have been on lesions of the luminal gut, there is increasing experimental and clinical evidence for its efficacy in cancer of the pancreas and biliary tract. With respect to BTC, the key questions for this uncommon malignancy are: (i) the value of adjuvant post-operative chemotherapy, and in those with irresectable disease, assessing the additional potential benefit of best supportive care plus (ii) PDT, (iii) single agent or combination chemotherapy, and/or (iv) local radiation, in improving survival. In the UK, the first three points are being addressed in a programme of clinical trials developed by the National Cancer Research Network Upper Gastro-Intestinal Group (www.ncrn.org), investigating (i) adjuvant gemcitabine vs surgey alone for resectable disease, and in those with locally advanced or metastatic BTC, (ii) PDT vs no PDT and (iii) gemcitabine vs gemcitabine and cisplatin.