

Gastrointestinal Cancers Symposium

General Session VI: Cancers of the Pancreas – Translational Research

Title: **Developing Novel Therapies for Pancreatic Cancer**

Speaker: Craig Logsdon, PhD
M.D. Anderson Cancer Center

Dr. Logsdon's talk covered four areas: 1) Introduction to pancreatic cancer, 2) Target discovery, 3) Preclinical validation, and 4) Clinical Trials.

1. Introduction to Pancreatic Cancer

- Pancreatic cancer has the worst prognosis of all major cancers and new therapies are desperately needed. There are two basic approaches to developing new cancer treatments:
 - **Develop Cancer Treatments via Molecules to Medicine:**
 - In molecules to medicine the drug comes first. The drug is found to have some anticancer activity, which is then studied to understand the mechanism.
 - Studies tend to be empirical. Dr. Logsdon stated that this approach has not been too successful, but he feels this is an approach that needs to be done.
 - **Develop Cancer Treatments via Targets to Treatments:**
 - In targets to treatment you find a molecule in cancer that you want to target/affect. The drug is developed and the target is validated.
 - Rational approach.

2. Therapeutic Target Discovery

- The typical approach to discovering an ideal therapeutic target involves genetic profiling. Currently available technology includes studying genetic mutations/amplifications, gene expression (transcript), and proteomics.
- No matter which technique is chosen, it requires rational comparisons. It is actually difficult to make these comparisons in pancreatic cancer for the following reasons:
 - A normal pancreas is primarily made up of acinar cells, occasional ducts and scattered Islets of Langerhans. In pancreatic cancer, the vast bulk of a tumor is made up of stroma.
 - When you compare pancreatic cancer to a normal pancreas you are comparing two vastly different things - acinar cell expression of a normal pancreas with stroma of pancreatic cancer.
 - Dr. Logsdon states they use chronic pancreatitis as an additional control in their lab because there is abundant stroma like that seen in pancreatic cancer. Comparing the two enables you to see what is expressed in the cancer cell. He believes chronic pancreatitis is a necessary control.
- Gene Expression
 - There are advantages/disadvantages in looking at targets expressed in cancer cells.
 - Advantages: specific for cancer.

- Disadvantage: tumors are heterogeneous. Not every cancer cell is the same so the target you find may not be contained in every cancer cell.
 - You can also aim for targets in the microenvironment.
 - An advantage is that targets in the microenvironment are homogenous and genetically stable.
 - A disadvantage is these targets are likely involved in normal physiology which is problematic when you aim to use them as targets.
 - Both EGFR and S100P molecules are found in cancer cells but also have effects in the microenvironment.
- The ideal therapeutic target is going to be highly specific for cancer (i.e. molecules that are mutated in cancer). This is the case with K-ras which is mutated in more than 90% of pancreatic cancer and not mutated in normal cells.
- Besides specificity another issue to consider is prevalence. The ideal therapeutic target should be present in a high proportion of specific tumors. Dr. Logsdon shared a couple of examples, comparing two targets in pancreatic cancer that his lab is working on: the EGFR and the molecule S100P.

The Ideal Target - Summary

	EGFR	S100P
Specific for cancer	No evidence for cancer specificity	Yes (~40 fold or more difference). No expression seen in normal pancreas cells and chronic pancreatitis
High proportion of tumors	Unclear, since EGFR is ubiquitous	Expressed in very few sites in the body, but is expressed in >94% tumors. Produced and secreted by PC cells
Critical function	Involved in cell growth, survival, motility and invasion	Increase cancer cell survival.

3. Preclinical Evaluation

- Issues to consider in preclinical evaluation:
 - Need to use a very specific method like molecular profiling when evaluating the target or therapy.
 - Tumors are heterogeneous.
 - No single cancer cell line or primary xenograft is sufficient.
 - Choice of cell model is critical.
 - Pancreatic tumors are resistant to several known treatments. Cell lines that are sensitive to failed treatments must be interpreted with caution for preclinical validation.
- Preclinical testing summary
 - Model characteristics are important.
 - Realistic cell lines are necessary.
 - Ideally, the target and drug should be tested separately.

- EGFR - preclinical support for effectiveness is limited to sensitive cells
- S100P - inhibition appears to increase killing of resistant cells

4. Clinical Trials

- Dr. Logsdon referenced the NCIC phase III clinical trial that showed a statistically significant impact on survival and resulted in the 2005 approval of erlotinib in combination with gemcitabine for unresectable locally advanced and metastatic pancreatic cancer. He stated that based on the data he presented to the audience he is surprised that this therapeutic combination showed any extension on survival.
- S100P has not been tested in clinical trials because there is lack of intellectual property for this molecule and a lack of financial support. Developing new analogs.

Summary - Comparison of EGFR and S100P as Targets:

EGFR	S100P
<ul style="list-style-type: none"> • Limited rationale to select EGFR as a target in pancreatic cancer. 	<ul style="list-style-type: none"> • S100P is excellent as a theoretical target.
<ul style="list-style-type: none"> • Poor support at the preclinical level but minor success in the clinic. 	<ul style="list-style-type: none"> • The preclinical data is positive although limited. Currently there is a lack of commercial interest.

Dr. Logsdon concluded this talk with the following points:

- Based on the theoretical criteria it should be possible to better select pancreatic cancer therapeutic targets.
- Preclinical evaluation of candidates can be improved by stringent criteria.
- Many potential targets are yet to be evaluated.

Title: Vaccines for Pancreatic Cancer
Speaker: Elizabeth Jaffee, MD
 Johns Hopkins University

Dr. Jaffe's presentation examined the rational and clinical data supporting the use and investigation of vaccines for the treatment of pancreatic cancer.

Vaccine development for cancer treatment raises new challenges:

- 1) Finding specific antigens to target in a specific tumor.
- 2) Identifying the most effective ways to immunize against those targets.
- 3) The interaction between the tumor and the immune system. The tumor seems to be able to turn off the immune system.

1. The First Challenge – Finding Specific Antigens to Target

- Need to find and target relevant tumor antigens that the immune system sees and recognizes.
- Johns Hopkins uses whole cell vaccines to identify clinically relevant tumor antigens.
- The characteristics of a candidate tumor antigen that constitutes a relevant tumor target include:
 - Antigens that are biologically important to tumor's progression.

- Immune response can be correlated with a clinical response.
- Targeting antigens that results in tumor regression or slows/stops tumor progression. Both results can aid patient's quality of life.

2. The Second Challenge – Identifying the Most Effective Way to Immunize Against a Target

- Developing methods for effectively delivering antigens for T-cell activation. This is where the field has made some progress over the past 10 years.
- The dendritic cell (DC) is a major antigen presenting cell that has to be targeted. There are a number of vaccine approaches that can target DCs including the following promising phase I and II trials:
 - 1) Recombinant poxviruses
 - 2) *Listeria monocytogenes* (bacteria that naturally targets the DC)
 - 3) Whole tumor cell expressing GM-CSF
 - 4) Pulsed DC cells
- In the past 5-6 years, the research community has learned that DCs express multiple signals for the optimal activation of T-cells.

3. The Third Challenge – The Interaction Between the Tumor and the Immune System

- Dr. Jaffe stated that the relation between the tumor and the immune system is where the research focus is presently.
- DCs not only activate T-cells, it can also down regulate them as well.
- The tumor microenvironment is very important because it induces immune suppressing signals.
- It is really complicated to activate the immune response and researchers are coming to an understanding of the signals important in this process.
- The overall immune response is a summation of the positive and negative signals provided to T-cells. The immunization is really just the first part of this; it provides multiple signals which results in the amplification of T-cells. The problem is there are a number of brakes/checkpoints of immune inhibition that come into play. An improved immune response correlates with clinical response.

Johns Hopkins' Research with GVAX

- GVAX is genetically modified tumor cells to express GM-CSF.
- Dr. Jaffe played a short animated video showing their vaccine approach that activates DCs.
- JHU's phase I trial (Protocol J9617) administered an allogenic GM-CSF vaccine in surgically resected PC patients. Patients also received adjuvant chemoradiation following vaccination, and a second, third and fourth vaccination followed each one month apart. Post vaccination DTH correlates with disease free survival.
- Looked at the 14 patients on study. They identified a number of candidate antigens. Mesothelin is identified as a candidate T-cell target. Mesothelin has:
 - Limited normal expression (mesothelial cells)
 - Expressed by 100% pancreatic cancers
 - Expressed by other cancers including ovarian, mesotheliomas, and non small-cell lung cancer (~30% of all cancers express mesothelin).

- The GVAX vaccine induced an immune response; there were also some interesting bioactivity in the immune responses:
 - Serum GM-CSF levels peak at 48 hours.
 - Local vaccine reactions with immune infiltration.
 - Systemic pruritic rashes 1-3 weeks after vaccine. This occurred in smaller group of patients and seemed to correlate with longer duration of disease free status.
 - Eosinophilia up to 25% within one week of vaccine
 - Recall induration (hardening of soft tissue), pruritus (itching), and erythema (redness) at old vaccine sites as long as nine years since the last vaccine.
- A phase II trial was conducted; the only difference was a vaccine booster was introduced at 6 months. Dr. Jaffee stated that the GVAX vaccine is safe and demonstrated bioactivity. The post vaccination induction of mesothelin-specific Tcell responses correlates with improved disease free survival.
- Additional boosts should be tested to evaluate improved median survival benefit.
- T-cell number alone is not the best predictor of clinical responses

Points to Consider

- The tumor's microenvironment is a dynamic process shaped by constantly changing genetic, inflammatory and vascular signals. For this reason, cancers require treatment with a combination of agents. Need more than one agent acting at a time.
- Preclinical models are confirming the synergy between vaccines and immune checkpoint inhibitors. This research is starting to go into phase I and II.

Where is vaccine therapy in 2007?

- We have potent dendritic cell based vaccines (GVAX, Listeriam fowlpox, etc.)
- Need more checkpoint inhibitors.
- Need more endpoints.
- More efficient and predictive trial designs are essential!
- More serum and T-cell markers needed.

Title: Predictive Markers for Gemcitabine Sensitivity in Pancreatic Cancer
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Speaker: James Farrell, MD UCLA, David Geffen School of Medicine
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- Dr. Farrell is a Gastroenterologist and spends a great deal of his time performing endoscopic ultrasound (EUS) in patients with pancreatic disease, including pancreatic cancer (PC).
- He explained that they have tried to go beyond just diagnosing PC and use EUS to guide the therapy and perhaps hopefully predict response to treatment.

Determining who will benefit from Gemcitabine

- Dr. Farrell explained the metabolism and uptake of gemcitabine. There are a number of transporters that get gemcitabine into the cells. The Human Equilibrative Transporter-1 (hENT1) is the predominant transporter for which gemcitabine gets into pancreatic cancer cells. hENT1 is found in normal pancreas tissue and in pancreatic cancer.
- There are several published studies that examined the role of hENT1 in pancreatic disease. Previous in vitro studies show hENT1 deficiency in pancreatic cells confers resistance to gemcitabine toxicity.

Smaller clinical studies have shown that hENT1 deficient pancreatic cancers by immunohistochemistry have a shorter survival despite gemcitabine chemotherapy.

- A more recent study published in 2006, looked at a larger group of patients both in the adjuvant and palliative setting. PC patients with high hENT1 gene expression had better overall survival in those that received gemcitabine.

Does research show that hENT1 is prognostic or predictive factor?

- To determine whether hENT1 has predictive value, Dr. Farrell and colleagues reviewed the phase III RTOG 9704 trial. This was an adjuvant treatment trial of resected PC. Patients were randomized into one of two arms; both arms received combinations of chemoradiotherapy. One arm received 5FU and one arm received gemcitabine. The trial did not show any statistical difference between the arms on this trial.
- Blood/serum and tissue samples were studied. Performed tumor tissue microarray in triplicate (used for immunohistochemistry purposes) and did high quality DNA for polymorphism work.
- Looked at hENT1 with respect to overall survival, between the Gemcitabine arm and 5FU arm.
- Dr. Farrell showed a series of survival curves looking at hENT1 immunohistostaining.
 - First looked at hENT1 and overall survival between the gemcitabine and 5FU arm. Compared the staining for hENT1 between high and low patients. Saw a statistically significant difference between patients with no hENT1 staining compared with those with low or high staining.
 - Those with no staining had median overall survival of 9.6 months whereas those with low or high staining had 19.2 months. There was no statistically significant difference between the low/high stain versus no stain groups in the 5FU arm.
 - Looked at different gradations of staining. Found statistically significant difference in median survival between low, high stain, and no stain. In the gemcitabine arm, they found the high stain group had median survival of 22.8 months. Low stain = 16.8 months, and no stain = 9.6 months.
 - This data suggests effect of hENT1 transporter with respect to its ability to confer survival advantage. They did not see a survival difference in the 5FU arm when looking at the three stain levels (high, low, and no stain).
- The RTOG9704 data showed that hENT1 is a predictive marker of response to gemcitabine in the adjuvant setting. Validation is required.
- It is Dr. Farrell's hope that in the near future a tumor biopsy obtained from a PC patient will be analyzed via gene expression or protein level, in combination with blood analysis for polymorphism work, and that you will be able to subtype these patients into different types of PC.
- Dr. Farrell concluded that he is encouraged by the talks by Drs. Logsdon and Jaffee earlier this morning, and that in the future we will be able to offer a variety of treatment options to PC patients.