Human equilibrative nucleoside transporter 1 (hENT1) in gemcitabine and FOLFOX (oxaliplatin, 5-fluorouracil and leucovorin) treated patients with metastatic pancreatic cancer: The randomized phase II PAN1 study.

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Background

The benefit of gemcitabine monotherapy in unselected advanced pancreatic cancer patients is only modest[1]. The addition of nab-paclitaxel to gemcitabine has recently been shown to improve survival compared to gemcitabine alone (median overall survival 8.5 months vs. 6.7 months; HR=0.72; 95%CI 0.62-0.83; p<0.001)[2]. Outcomes were also improved by the non-gemcitabine containing FOLFIRINOX (5FU, Oxaliplatin, Irinotecan) combination (median overall survival 11.1 months vs. 6.8 months for gemcitabine alone; HR=0.57; 95%CI 0.45-0.73; p<0.001) in a similar group of patients in the PRODIGE4/ACCORD11 randomised trial[3]. Toxicity was increased by both combination regimens, particularly in the case of FOLFIRINOX with 42.5% of patients requiring growth factor support in that arm of the study[3].

The combination of oxaliplatin and infusional 5FU also appears to be active and well tolerated in phase II trials in previously untreated metastatic pancreatic cancer[4,5]. Following gemcitabine failure, this doublet is superior to best supportive care, and to 5FU alone[6,7], and may therefore be a viable treatment alternative in the first line setting.

After more than a decade of gemcitabine monotherapy as the only treatment for this disease, patients now have options for treatment, including gemcitabine and non-gemcitabine containing regimens.

Tools are needed to select between these divergent treatments, and better tolerated regimens are required for patients who may not be suitable for these novel combinations. It is of particular interest to evaluate the oxaliplatin-5FU combination in the patients whose biomarker characteristics predict for less benefit from gemcitabine treatment.

Biomarker-based selection of cancer treatments has already entered routine clinical practice in some other cancer types, but not yet in pancreatic cancer. The human equilibrative nucleoside transporter 1 (hENT1), a member of the SLC29 family of integral membrane proteins which is involved in the transport of purine and pyrimidine nucleosides across the cell membrane, has shown promise as a predictive marker of benefit from gemcitabine[8 – 11] . Under expression of hENT1 may explain the reduced efficacy of gemcitabine in some patients.

Thus far studies of hENT1 in pancreatic cancer have yielded contradictory results. When tested retrospectively in the large cohorts of patients from the RTOG9704 and ESPAC trials of resected pancreatic cancer hENT1 status appeared to correlate with a benefit from gemcitabine, but not from 5-fluorouracil, consistent with the hypothesis it is a predictive marker and not a prognostic one[8,12] . However this has not subsequently been replicated in a prospective study in metastatic disease with a more recently developed hENT1 assay, in which the test used in the trial did not seem to be able to predict the outcome of gemcitabine treated patients[13].

Methods

PAN1 was a randomized phase II study of gemcitabine or FOLFOX (oxaliplatin, 5-fluorouracil and folinic acid) in previously untreated advanced pancreatic adenocarcinoma tested prospectively for hENT1. Initially the study only included patients with metastatic disease and hENT1 testing was required prior to randomization, but in June 2012 the study protocol was amended to include patients with locally advanced disease and to allow hENT1 testing after randomization.

Primary objective: To prospectively evaluate hENT1 as a predictive marker of benefit from gemcitabine treatment in locally advanced and metastatic pancreatic cancer.

Primary Endpoint: Progression free survival (PFS)

Secondary Endpoints:

i) Efficacy/activity of gemcitabine and FOLFOX will be assessed by:
- Overall survival (OS)
- Response rate according to RECIST v1.1
- CA19.9 response

ii) Treatment related toxicity in each chemotherapy group according to CTCAE (Common Terminology Criteria for Adverse Events) v4.0;

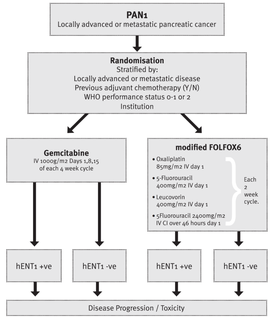
iii) Establish a tissue bank from patients treated with and without gemcitabine to support further biomarker studies.

Eligibility criteria:
- Males or females with histologically confirmed pancreatic adenocarcinoma that is either:
  - Metastatic or
  - Locally advanced and not suitable for upfront chemoradiation.
- Eligibility of patients with newly suspected disease (i.e. no prior histological confirmation) must be confirmed by core biopsy prior to randomisation. Radiological evidence of disease recurrence is adequate for previously resected patients.
- No previous treatment, except:
  - If adjuvant systemic therapy was received following resection, study entry is permissible if disease recurrence has occurred at least 6 months after completion of chemotherapy.
  - Previous radiotherapy is permissible if disease progression has occurred outside the radiotherapy field and disease recurrence has occurred at least 6 months after completion of radiotherapy
  - Previous chemoradiotherapy is permissible if only radiosensitiser dose chemotherapy was used and disease progression has occurred outside of the radiotherapy field at least 6 months after completion of treatment.
- Informed consent for all trial procedures
- WHO performance status 0-2.
- Adequate renal, hepatic and haematological function
- Absence of peripheral neuropathy of any cause, of grade 2 or worse by CTCAE v4.0.

Statistical considerations: At least 40 patients were required to receive gemcitabine to demonstrate a minimum difference in 4-month progression free survival from 25% to 53% between the hENT1 negative and hENT1 positive groups with 80% power and 95% confidence.

hENT1 immunohistochemistry and scoring: hENT1 immunohistochemical staining and scoring was performed as previously described [Marechal Clin Cancer Res 2009]. The secondary antibody was goat anti-mouse conjugated to a horseradish-peroxidase-labelled dextran polymer (Dako En Vision+) purchased from DAKO Corporation. Slides of formalin-fixed, paraffin-embedded tumour tissue from study patients were sent to Cross Cancer Institute, Edmonton, Alberta, Canada for hENT1 staining. Quantitative scoring using light microscopy was performed by an experienced pathologist blinded to clinical characteristics and outcome, and only on invasive adenocarcinoma cells. Intensity of hENT1 staining was scored according to: 0=no staining, 1=weakly positive, 2=moderately positive, and 3=strongly positive. The percentage of adenocarcinoma cells staining at the different intensity levels was recorded and a composite final score (ranging between 0-300) was determined by multiplying the intensity score and the percentage of the specimen. A final score of = or >80 was considered as hENT1 high.

Study Schema



All patients received their assigned treatment for at least 4 months, or until progression or unacceptable toxicity. After 4 months, patients with locally advanced disease who had not progressed, in whom chemoradiation was considered appropriate could cease study treatment and proceed to chemoradiotherapy with a concurrent fluoropyrimidine as per standard local practice. Patients with locally advanced disease considered unsuitable for chemoradiation continued on study treatment until progression or unacceptable toxicity.

RESULTS: A total of 16 patients were recruited from across 9 Australia sites between April 2012 and February 2013 when the study was closed due to poor accrual. Factors which may have been barriers to recruitment include:
- competing industry sponsored trials;
- study treatments which may have been less appealing due to the emergence of data for new combination regimens and lack of inclusion of novel agents;
- poor performance status of potential patients; and,
- difficulties accessing adequate suitable tumour tissue in a timely fashion.

Only 1 patient had locally advanced disease. 1 patient in the FOLFOX arm who died before study treatment was excluded from the outcome analysis.

	Gemcitabine (N=7)	FOLFOX (N=9)
hENT1 high	4 57.1% (95%CI 25.1-84.2%)	3 33.3% (12.1-64.6%)
hENT1 low	3	6

hENT1: hENT1 status was available for all study patients. 7 out of 16 patients were hENT1 high (43.8%, 95%CI 23.1-66.8%). The patient in the FOLFOX arm who died before study treatment was hENT1 low.

Baseline Demographics

		Gemcitabine (N=7)	FOLFOX (N=8)
		n (%)	n (%)
Disease	**Locally advanced**	0	1 (12.5%)
	Metastatic disease	7 (100.0%)	7 (87.5%)
Age	**Median**	66.4 years	69.0 years
	Range	56-73	62-76
Gender	**Male**	3 (42.9%)	4 (50.0%)
	Female	4 (57.1%)	4 (50.0%
Previous resected primary		2 (28.6%)	2 (25.0%)
Previous adjuvant treatment		1 (14.3%)	1 (12.5%)
Performance status	**0**	4 (57.1%)	7 (87.5%)
	1	3 (42.9%)	1 (12.5%)
hENT1 high		4	3

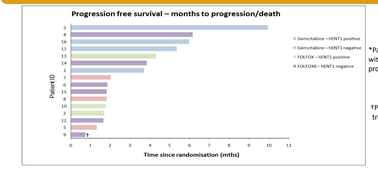
Survival and treatment responses

	Gemcitabine			FOLFOX		
	All (N=7)	hENT1+ (N=4)	hENT1- (N=3)	All (N=8)	hENT1+ (N=3)	hENT1- (N=5)
Median PFS, months	3.7 (95%CI 1.3-6.0)	5.7	1.8	1.8 (1.7-4.3)	1.8	1.9
Median OS, months	7.3 (95%CI 1.3-13.9)	7.3	3.5	5.4 (1.7-11.7)	4.3	6.4
Partial response (%)	1 14.3%	1	0	2 25.0%	1	1
Stable disease (%)	3 42.9%	3	0	1 12.5%	0	1
Progressive disease (%)	2 28.6%	0	2	4 50.0%	1	3
Not assessable (%) *	1 14.3%	0	1	1 12.5%	1	0

The analysis was updated in November 2013, with only 2 patients still alive. The median overall survival for all patients was 5.9 months.

*** Not assessable**: Patients 2 and 5 died prior to first scan.

Progression free survival



*Patients 2, 5, 9 and 13 died without documented progression

†Patient 9 received no protocol treatment

Overall Survival

*Patients 11 and 15 alive at last follow-up

†Patient 9 received no protocol treatment

Time to progression and death

*Patients 2, 5, 9 and 13 died without documented progression

†Patients 11 and 15 alive at last follow-up

‖Patient 9 received no protocol treatment

Adverse events

	Grade III/IV	
	Gemcitabine (N = 7)	mFOLFOX6 (N =8)
Symptoms/Adverse event	n	n
Any adverse event graded 3+	5	5
Nausea	0	2
Vomiting	0	1
Constipation	1	0
Fatigue	0	1
Neutropaenia	1	3 †
Elevated GGT	1 ‡	1

† 1 event was Grade 4
‡ Grade 4

3 patients (42.9%, 95%CI 15.8-75.0%) in the gemcitabine arm and 5 in the FOLFOX arm (62.5%, 95% CI 30.6-86.3%) had at least 1 dose reduction.

End of treatment information

		Gemcitabine (N=7)	FOLFOX (N=8)
Number of treatment cycles commenced, median		4	4
Reasons for ceasing treatment	Confirmed tumour progression	5	6
	Clinical progression	0	1
	Death	1	1
	Clinician preference	1	0

Conclusion

- The small number of patients limits the conclusions which can be drawn from this study. Any statistical comparison of the different subgroups of patients is not possible but the results are potentially still interesting for hypothesis generation.
- A numerically longer survival (PFS and OS) was observed in advanced pancreatic cancer patients receiving gemcitabine who were hENT1 high compared to those who were hENT1 low. The outcomes of hENT1 high and low patients treated with FOLFOX was more similar. This may well have been due to chance, but these observations are consistent with the hypothesis that hENT1 may be a predictive factor for benefit from gemcitabine, but is not otherwise a prognostic factor for outcome in pancreatic cancer.
- These results support further evaluation of hENT1 in this setting, although a more contemporary study design in advanced pancreatic cancer may be to randomize patients between gemcitabine plus nab-paclitaxel or FOLFIRINOX.
- hENT1 should also be studied prospectively as a means of selecting between gemcitabine and 5FU adjuvant treatment in resected disease.
- The outcome of the overall FOLFOX treated group appeared to be poorer than expected. It is unclear whether this may be due to the regimen being less active than anticipated or whether other factors such as toxicity were important.
- The poor accrual does demonstrate the difficulties conducting clinical trials in this disease and the challenges which are likely in implementing up front biomarker testing in a disease in which obtaining suitable tumour tissue for testing is problematic. A practice shift away from fine needle aspiration in favour of core biopsies needs to occur, but in the longer term there is a need for biomarker testing which can be performed on minimal tissue.

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