Liverpool Experimental Cancer Medicine Centre

Achievements 2018 – 2019

The Clatterbridge Cancer Centre NHS Foundation Trust

CANCER RESEARCH UK Liverpool Cancer Trials Unit

UNIVERSITY OF LIVERPOOL

ecmc Liverpool

Development of Digital IHC Approaches

Background

Automated scoring using digital pathology software offers a promising alternative to preclude these hurdles, however the heterogeneity of PDAC and its corresponding stroma can be challenging for digital approaches to overcome.

Methods

Pancreatic ductal adenocarcinoma TMAs were stained for hENT1 by immunohistochemistry. Cytoplasmic staining in tumour cells were scored by a specialist histopathologist, on a four-point scale: negative, weak, moderate, strong. Where heterogeneous staining was noted, more than one score was given.

A random forest tissue classification algorithm was subsequently constructed using QuPath digital pathology software (https://qupath.github.io/), classifying tumour cells from the tumour microenvironment in a training subset of TMA cores. Cells were classified on a four-point scale from 0-3, and H-scores calculated for each core.

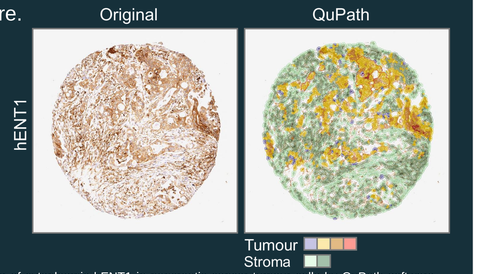

Figure 1. Scoring of cytoplasmic hENT1 in pancreatic cancer tumour cells by QuPath software. Shown is a core where hENT1 expression is observed in both the tumour and stromal compartments (left), with the classification algorithm able to separate tumour from the surrounding tumour microenvironment and score individual cells by hENT1 expression (right).

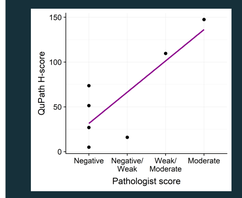

Figure 2. Comparison of manual scores by a specialist histopathologist with H-scores calculated by digital pathology software in a training

Results

Initial classification of the training set of TMA cores revealed good separation of tumour cells from the surrounding microenvironment, even in cores with challenging IHC staining (Figures 1 & 2).

Potential to standardise and streamline IHC-based biomarker assays

Biomarker Based Choice of Combination Therapies in Pancreatic Cancer

Background

Adjuvant chemotherapy for pancreatic cancer with gemcitabine (often in combination with capecitabine) or 5-fluorouracil (5-FU, often as part of the FOLFIRINOX regimen) improves long-term survival post resection. At present there are no biomarkers available to stratify treatment for patients with pancreatic cancer (PDAC). Equilibrative nucleoside transporter 1 (hENT1) has greater affinity for gemcitabine than 5-FU.

High hENT1 expression is associated with improved survival in patients with gemcitabine. Intracellular deamination of gemcitabine by cytidine deaminase (CDA) increases efflux and inactivation of the drug (Figure 3).

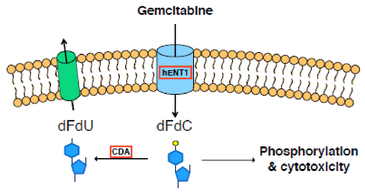

Figure 3. Simplified schematic of the role of hENT1 and CDA in the membrane transport and inactivation of gemcitabine.

Results

CDA mRNA and protein expression varied between patients (Figure 4A-E) but did not correlate (Figure 4F).

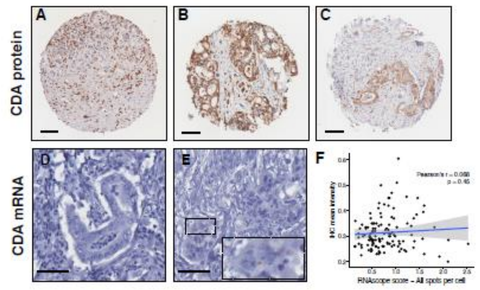

Figure 4. Examples of PDAC TMA cores histologically stained for CDA protein and mRNA. Protein staining was heterogeneous, with expression varying from no tumour expression (**A**) to strong (**B**) to moderate (**C**) intensity. mRNA expression was similarly heterogeneous, with examples seen of no expression (**D**) and high expression (**E**). **F –** CDA protein and transcript as measured digitally did not correlate. Scale bars = 100µm (**A, B, C**) and 50µm (**D**, **E**).

Patients with low hENT1 protein did better with 5-FU only if CDA was low (Table 1/Figure 5).

CDA transcript improves hENT1 prediction of response to gemcitabine in pancreatic cancer

This work underpinned a collaboration with NuCana to investigate a novel gemcitabine-based protide for patients with pancreas cancer.

Table 1. Median overall survival in biomarker subgroups split by treatment arm

Arm	Biomarker expression (high or low)	Number	Median OS	95% confidence interval	Log rank	*P*-value
5-FU/FA	CDA High	36	14.6	8.4-24.1	5.17	**0.0229**
	CDA Low	96	26.4	21.4-29.7		
GEM	CDA High	56	21.2	15.7-26.2	5.14	**0.0234**
	CDA Low	89	24.8	18.3-33.0		
5-FU/FA	hENT1 High	59	22.6	17.3-28.6	0.53	0.4658
	hENT1 Low	69	24.1	15.9-30.4		
GEM	hENT1 High	82	26.0	21.2-32.8	7.58	**0.0059**
	hENT1 Low	58	16.8	14.1-24.8		
5-FU	CDA Low, hENT1 Low	44	29.3	21.9-41.9	6.14	0.1050
	CDA High, hENT1 Low	25	14.2	7.9-24.1		
	CDA Low, hENT1 High	49	22.6	16.9-29.6		
	CDA High, hENT1 High	10	20.1	5.0-37.5		
GEM	CDA Low, hENT1 Low	34	18.3	13.9-28.3	12.0	**0.0073**
	CDA High, hENT1 Low	24	14.6	11.1-25.1		
	CDA Low, hENT1 High	52	28.0	21.1-45.5		
	CDA High, hENT1 High	30	23.8	16.6-28.7		

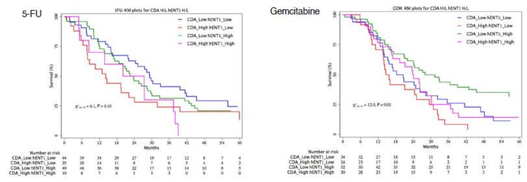

Figure 5. Kaplan-Meier analysis for combined CDA mRNA and hENT1 protein subgroups.

Overcoming Challenges in Toxicity Based Sampling

Background

The HYST study utilises a systems pharmacology multiomic approach to investigate immunological mechanisms of checkpoint related toxicity.

Analysis pathways include:

- Genetic analysis to determine a relationship between HLA type and irAEs.
- Immune focussed transcriptomics.
- Cellular analysis using mass cytometry and in-vitro T-cell assays.
- The relevance of recognised cytokines and autoantibodies.

Identification and recruitment of participants and sample collection have been challenging due to multi-centre treatment of toxicities and varied sample collection schedules (Figure 6).

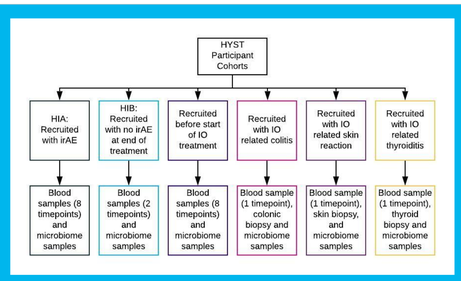

Figure 6. HYST recruitment cohorts and sample collection schedules

Implementation of Process Change

- Identification of participants through list generated by Clinical Effectiveness Team of individuals prescribed immunotherapy.
- Production of database and bespoke web-based sample tracker with customised reports.
- Weekly multi-disciplinary cross centre teleconferences for recruitment and follow up planning.
- Development of weekly skin toxicity clinic with addition of dermatologist to team.
- Establishment of links with IO nurse specialist.
- Central contact point for research visit organisation.

Increased recruitment rate, improved compliance with sample collection schedule and enhanced data integrity.

Contact Us

If you require any further information please get in touch:

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We would like to invite you to a workshop in September 2019 to discuss becoming involved in the HYST study and potential translational collaborations. If you are interested in attending please contact us.

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CHIEF SCIENTIST OFFICE

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