Soluble immune checkpoint receptor landscape in liver metastases of neuroendocrine neoplasms: a new perspective for immunotherapy

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Introduction

- Patients with neuroendocrine liver metastases (LM-NEN) have poor survival rates and limited therapeutic options.
- Targeting immune cell checkpoint receptors (CRs), such as programmed death 1 (PD-1), with checkpoint inhibitors has revolutionised the field of oncology. However, results suggest that only a limited number of NEN patients will benefit from anti-PD-1 therapy, as the tumours do not display significant positivity for the PD-1 ligand (PD-L1).
- Recent evidence has described an extensive network of soluble (cell-free) CRs beyond PD-1 that act in concert to not only inhibit but also stimulate anti-tumour immunity. The function of these inhibitory and stimulatory soluble CRs in NENs is unknown and unexplored.

Aims

1. Characterise the soluble CRs landscape in the plasma of patients with LM-NEN.
2. To investigate whether the source of soluble CRs is the NEN tumour tissue using a novel human immunocompetent organotypic tissue slice model for LM-NENs established in our lab.

Methods

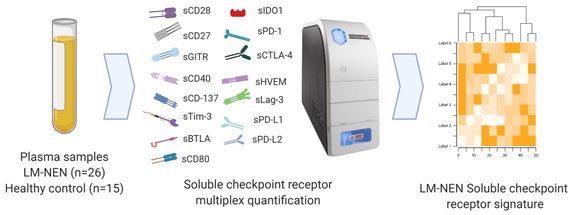
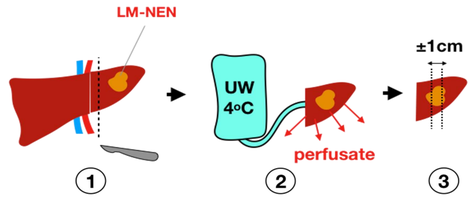


Figure 1. Plasma samples from patients with LM-NEN (n=26) and healthy control (n=15) were collected. A panel of 15 Soluble checkpoint receptors including sBTLA; sCD27; sCD28; sCD40; sCD80; sCD137; sCTLA-4; sGITR; sHVEM; sIDO-1; sPD-1; sPD-L1; sPD-L2 were simultaneously measured by multiplex Luminex technology using a custom-made checkpoint panel.



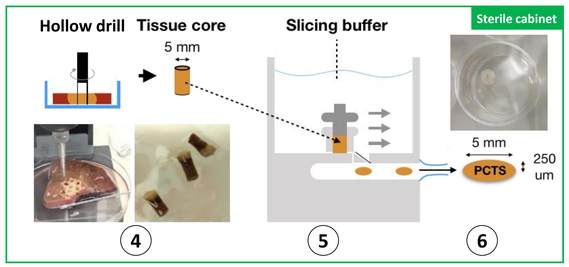


Figure 2. Preparation of LM-NEN precision cut tissue slices (PCTS). Resected liver tissue is obtained from post-surgery (1) and flushed with ice cold UW (cold storage) solution through open hepatic veins and arteries (2). A piece of +-1 cm thick tissue containing tumour and surrounding background liver tissue (3) is transported to the laboratory. The tissue is cored using a hollow drill bit (4). The cores are sliced using a sterile tissue microtome (5) and slices are collected in ice cold UW solution. After all PCTS (6) have been prepared incubation with orbital shaking in a hyperoxic environment. Medium collected and changed daily. Abbreviations: UW, University of Wisconsin; KBH, Krebs Henseleit buffer; PCTS, Precision cut tissue slices.

Results

		LM-NEN (n=24)	Healthy Control (n=15)
Gender	Male	12 (46.2%)	5 (41.7%)
	Female	14 (53.9%)	7 (58.3%)
Age Range in Years (Mean)		36-81 (60.0)	24-50 (34.1)
Ethnicity	Caucasian	23 (88.5%)	
	Asian	1 (3.9%)	
	Black/African/ Caribbean	1 (3.9%)	
	Other/ Mixed	1 (3.9%)	
Primary Tumour	Bowel NEN	14 (53.9%)	
	Pancreatic NEN	9 (34.6%)	
	NEN of Other Origin	3 (11.5%)	
Histology Grade of Tumour	G1	7 (26.9%)	
	G2	10 (38.5%)	
	G3	0 (0%)	
	Unassessed	9 (34.6%)	

Table 1. Patient characteristics for the plasma samples. Plasma samples were obtained from 24 patients with LM-NEN and 15 Healthy controls.

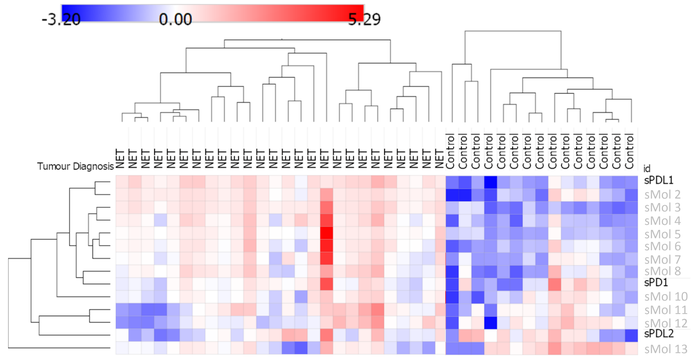


Figure 3. Hierarchical clustering analysis for levels of soluble checkpoint receptors in plasma samples from LM-NEN compared to healthy controls. A clear differentiation in the soluble checkpoint receptor signature can be observed between patients with LM-NEN and healthy controls. These data reveal that soluble checkpoint receptors beyond PD-1, PD-L1 and PD-L2 are dysregulated in patients with LM-NEN.

Patient	Sex	Age	Ethnicity	Primary	Treatment	Grade	Ki67	Fibrosis	Chromo-granin A
15	M	81	Caucasian	Lung	Hydroxy carbamide	G2	8	F0	+
45	M	58	Caucasian	Pancreatic	Octreotide	G2	16.9	F0	+
51	F	51	Caucasian	Small Bowel	-	G2	11	F0	+
62	F	52	Caucasian	Pancreatic	Streptozocin/ Capecitabine	G1	3	F1-F2	+
77	M	70	Caucasian	Small Bowel	Lanreotide	G2	3.6	F0	+
106	F	69	Caucasian	Small Bowel	Lanreotide, Octreotide	G2	4.6	F0	+

Table 2. Clinical characteristics for the resected LM-NEN tissue samples used for PCTS. Liver metastasis from NEN-tumours were obtained from 6 patients with primary tumour in the gastroenteropancreatic tract (n=5) or in the lung (n=1). All tumour samples were positive for neuroendocrine differentiation marker Chromogranin A. All tumours were low or intermediate grade (G1-G2).

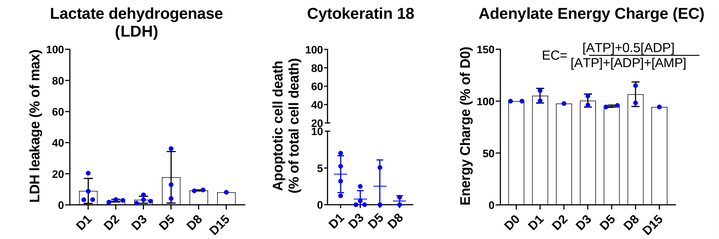


Figure 4. Precision cut tumour slices viability is maintained over extended culture. Lactate dehydrogenase (LDH), released upon cell death was consistently low. Apoptotic specific death measured by full length cytokeratin 18 (M65 ELISA, total death) and caspase cleaved CK18 (M30 ELISA, apoptosis specific). Metabolic capacity was measured by quantifying the intracellular ATP, ADP and AMP levels and calculating the energy charge (EC).

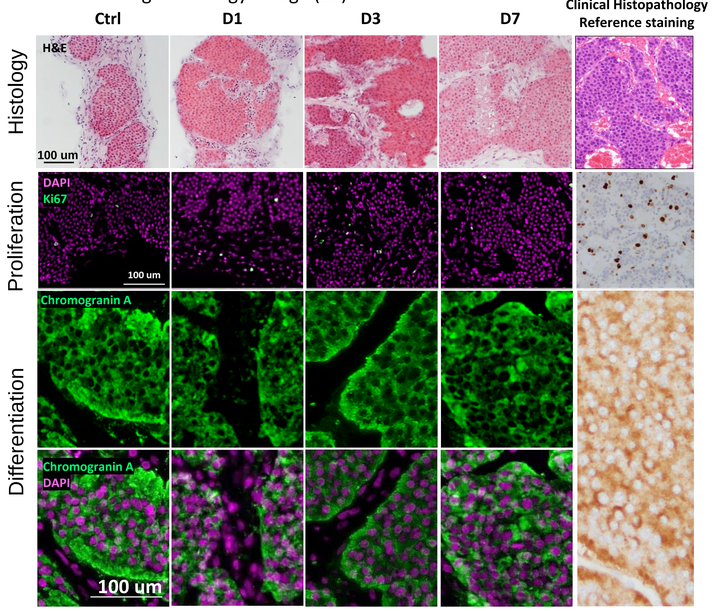


Figure 5. Patient specific histological characteristics are maintained over duration of the culture as compared to clinical histopathology staining (right panel). Haematoxylin and Eosin staining (top panel), proliferation capacity by Ki67 staining (middle) and neuroendocrine differentiation marker Chromogranin A (bottom panel).

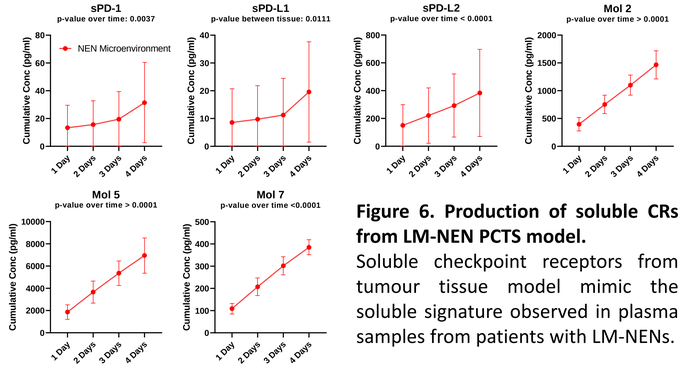


Figure 6. Production of soluble CRs from LM-NEN PCTS model. Soluble checkpoint receptors from tumour tissue model mimic the soluble signature observed in plasma samples from patients with LM-NENs.

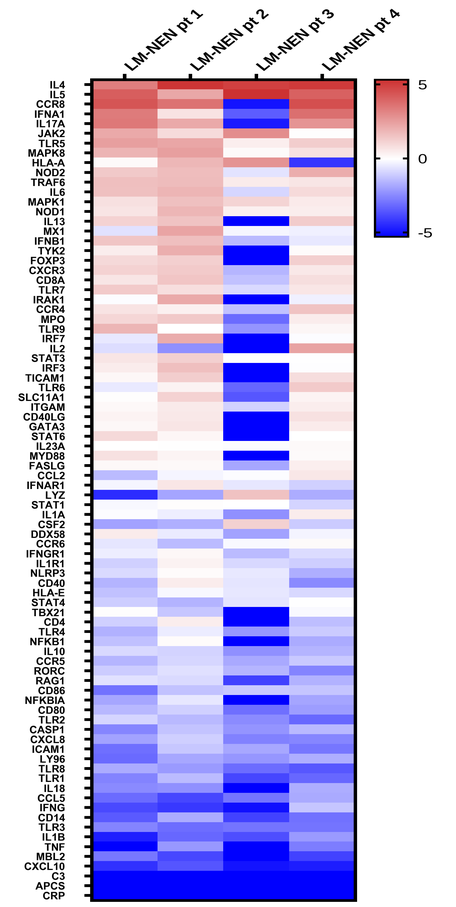


Figure 7. PCR microarray of innate and adaptive immunity markers in LM-NEN tumour tissue slices. Patient heterogeneity and individual characteristics are maintained in the PCTS model, including the distinct features of immune cell infiltration.

Conclusions

1. **There is a disease specific soluble CR landscape in patients with LM-NENs which includes both stimulatory and inhibitory soluble checkpoints.**
2. **LM-NEN Precision Cut Tumour Slice model retains tumour specific and immunological characteristics for up to 15 days and actively produces the distinct soluble CRs signature observed in the plasma.**

References: Palma E, Doornebal EJ, Chokshi S, ''Precision-cut liver slices: a versatile tool to advance liver research'' Hepatology international, (2018)

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