1687P - Targeted metabolomics reveals dynamic changes and potential therapeutic targets in the serum metabolome of patients receiving adoptive cell therapy with tumor infiltrating lymphocytes

Luke Mantle [1,2], Douglas Millar, Celine Robert-Tissot, Robert Laister, Valentin Sotov, Linh Nguyen, Maysa Vilbert [1,2], Thiago P. Muniz [1,2], Ian Hirsch [1,2], Mauricio Ribeiro [1,2], Erica C. Koch [1,2], Pamela Ohashi, **Marcus Butler** [1,2] and Samuel Saibil [1,2]

1Department of Medical Oncology and Hematology, Princess Margaret Cancer Centre, University Health Network, Toronto, Canada

2Department of Medicine, Division of Medical Oncology, University of Toronto, Toronto, Canada

BACKGROUND

- Adoptive cell therapy (ACT) using tumor-infiltrating lymphocyte (TILs) has shown clinical responses in the treatment of metastatic melanoma.
- Emerging data has demonstrated that levels of circulating metabolites significantly affect T cell function and survival in multiple pre-clinical models of ACT.
- Few studies have examined the circulating metabolome in patients undergoing ACT.
- We aimed to investigate changes in the serum metabolome in patients with metastatic melanoma undergoing ACT with TIL.

METHODS

- Samples were obtained longitudinally from 9 patients with metastatic cutaneous melanoma undergoing ACT with TILs.
- Sample A was obtained prior to lymphodepletion, sample B was collected after lymphodepletion one hour prior to TIL infusion, sample C was obtained 24 hours post TIL infusion and sample D was collected 4 weeks post infusion.
- Serum metabolites were measured using targeted mass spectrometry and differences in metabolites were compared using Random Forest, Multidimensional scaling analysis and the U-Mann-Whitney test.

RESULTS

- Comparison between samples A and B revealed a significant increase only in ortho-hydroxyphenylacetic acid.
- Comparison of samples B to C showed significant decrease in multiple metabolites including Lysophosphatidyl choline (LPC) 16:0 and LPC 18:0.
- Comparison of sample C to D showed a significant decrease of ortho-hydroxyphenylacetic acid and increase in LPC 16:0 and LPC 18:0.
- In *ex vivo* experiments, human T cells pretreated with LPC 18:0 demonstrated increased proliferation and IL-2 production in a dose dependent manner.

CONCLUSIONS

- ACT with TILs caused dynamic changes in circulating metabolites. Most significantly, a decrease in LPC after TIL infusion suggesting LPC uptake by the infused T cells.
- LPC has been shown to be involved in CD8+ memory T cell maintenance. Exposure to LPC pre-infusion may potentially improve infused CD8+ T cell IL-2 production and engraftment warranting further investigation.

Patient	Sex	Age	M stage	Previous Treatment
1	M	43	M1b	None
4	M	64	M1c	Ipi/Nivo, Carbo-tax
7	F	35	M1c	Carbo-tax, Ipi
16	M	48	M1c	Dabrafenib/Trametinib, Ipi, Pembro
22	M	49	M1c	Ipi, Pembro
19	M	35	M1c	DTIC, Ipi, Pembro, Carbo-tax
29	F	34	M1c	DTIC, Ipi, Pembro, IL-2 (injections)
8	M	61	M1c	Ipi/Nivo, Pembro
9	M	61	M1c	Nivo, anti-PD-1/anti-GITR, Carbo-tax

Table 1: Patient characteristics from 9 cutaneous melanoma patients treated with tumor-infiltrating lymphocytes.

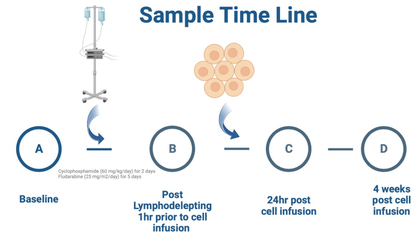

Fig 1: Representation of when serum metabolic samples were obtained. Sample A (baseline), B post lymphodepletion 1hour prior to cell infusion, C 24 hours post infusion, D 4 weeks post infusion

A vs B

Metabolite	t.stat	p.value	`-LOG10(p)`	FDR
Ortho-Hydroxyphenylacetic acid	-5.3	7.5E-05	4.1	5.7E-03
Citrulline	5.2	8.2E-05	4.1	5.7E-03
Hypoxanthine	4.9	1.6E-04	3.8	7.4E-03

T-Test FDR < 0.1

B vs C

Metabolite	t.stat	p.value	`-LOG10(p)`	FDR
LysoPC(16:0)	3.0	8.3E-03	2.1E+00	0.8
Uridine	2.7	1.7E-02	1.8E+00	0.8
LysoPC(18:0)	2.6	2.0E-02	1.7E+00	0.8
Chenodeoxyglycocholic acid	-2.5	2.2E-02	1.7E+00	0.8
5'-Methylthioadenosine	-2.4	3.0E-02	1.5E+00	0.8

T-Test Raw p-value < 0.05

C vs D

Metabolite	t.stat	p.value	`-LOG10(p)`	FDR
Ortho-Hydroxyphenylacetic acid	9.6	4.7E-08	7.3	6.6E-06
Citrulline	-6.5	7.6E-06	5.1	4.0E-04
LysoPC(16:0)	-6.4	8.5E-06	5.1	4.0E-04
LysoPC(18:0)	-5.0	1.3E-04	3.9	4.7E-03
Glycine	-4.5	3.6E-04	3.4	1.0E-02
L-Lysine	-4.2	7.1E-04	3.2	1.6E-02
L-Histidine	-4.1	8.1E-04	3.1	1.6E-02
L-Threonine	-3.7	1.9E-03	2.7	3.4E-02
LysoPC(24:1(15Z))	-3.6	2.5E-03	2.6	3.5E-02
Linoleyl carnitine	-3.6	2.5E-03	2.6	3.5E-02
Methylcysteine	-3.5	2.8E-03	2.6	3.6E-02

T-Test FDR < 0.05

Fig 2: False discovery rate for serum metabolites from samples A vs B, B vs C and C vs D

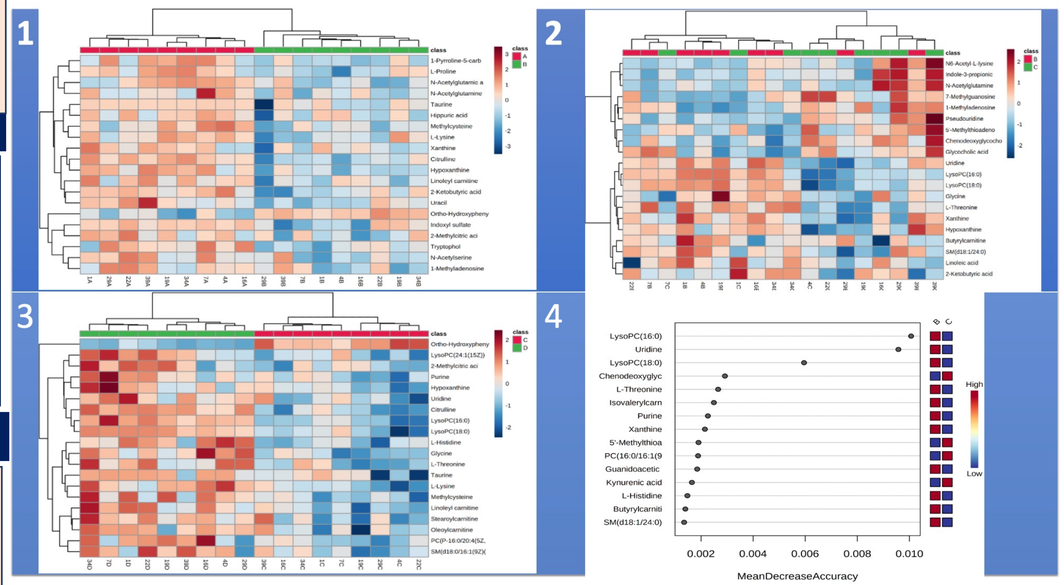

Fig 3: PANEL (1) Heatmap Top 20 metabolites A vs B, (2) Heatmap for B vs C, (3) Heatmap C vs D, (4) Random Forest B vs C.

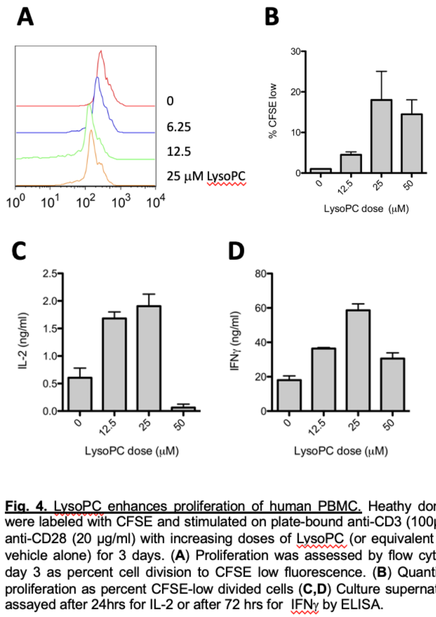

Fig. 4. LysoPC enhances proliferation of human PBMC. Heathy donor PBMC were labeled with CFSE and stimulated on plate-bound anti-CD3 (100µg/ml) and anti-CD28 (20 µg/ml) with increasing doses of LysoPC (or equivalent dilution of vehicle alone) for 3 days. (**A**) Proliferation was assessed by flow cytometry on day 3 as percent cell division to CFSE low fluorescence. (**B**) Quantification of proliferation as percent CFSE-low divided cells (**C,D**) Culture supernatants were assayed after 24hrs for IL-2 or after 72 hrs for IFNγ by ELISA.

Contact: luke.mantle@uhn.ca