A Serum Metabolomic Fingerprint of Bevacizumab and Temsirolimus Combination as First-Line Treatment of Metastatic Renal Cell Carcinoma

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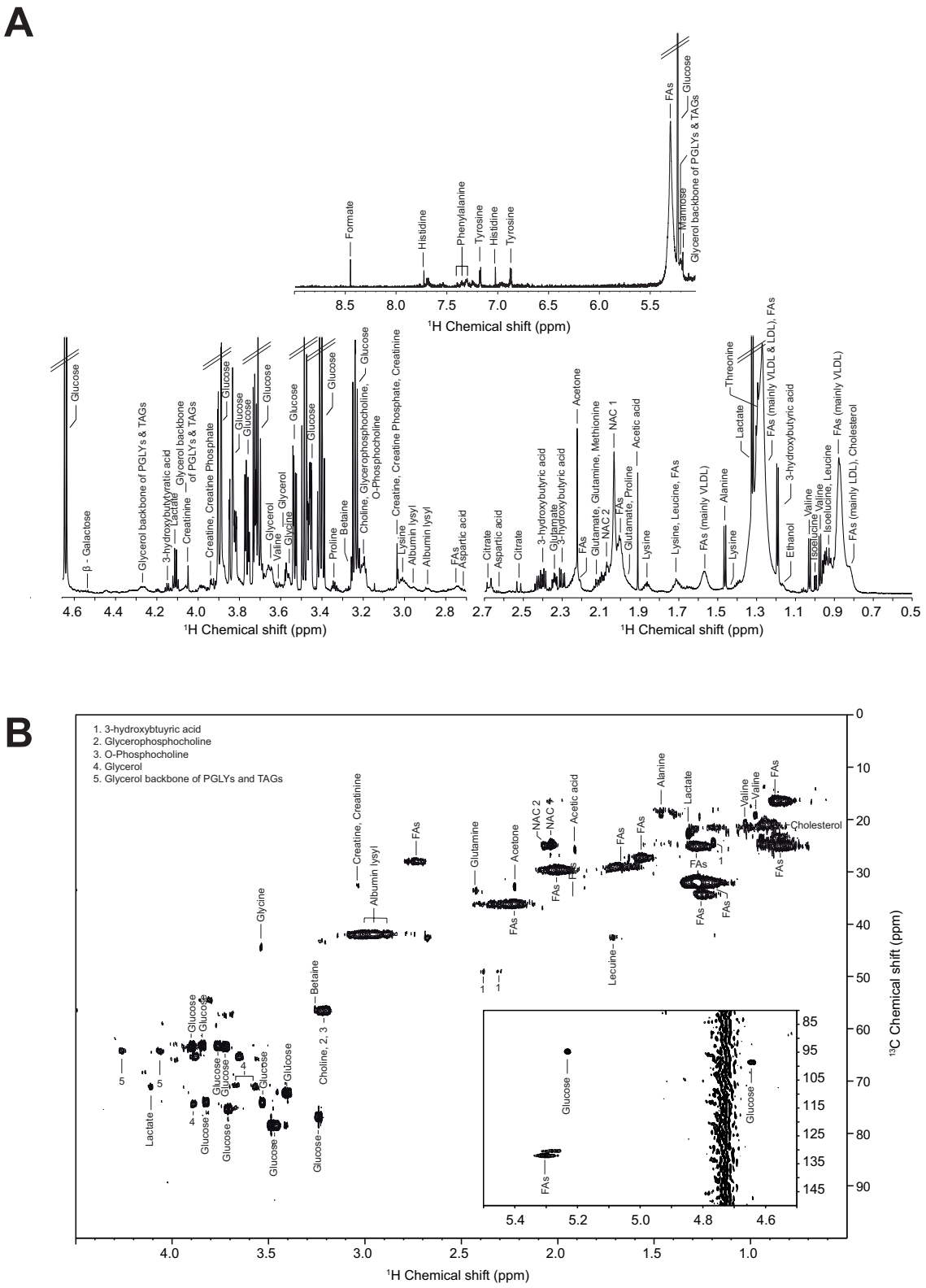


Supplementary Figure 1: Representative ^{1}H CPMG spectrum (A) and a ^{1}H -^{13}C NMR HSQC spectrum (B) at 800 MHz for a mRCC cancer patient treated with the temsirolimus and bevacizumab combination.

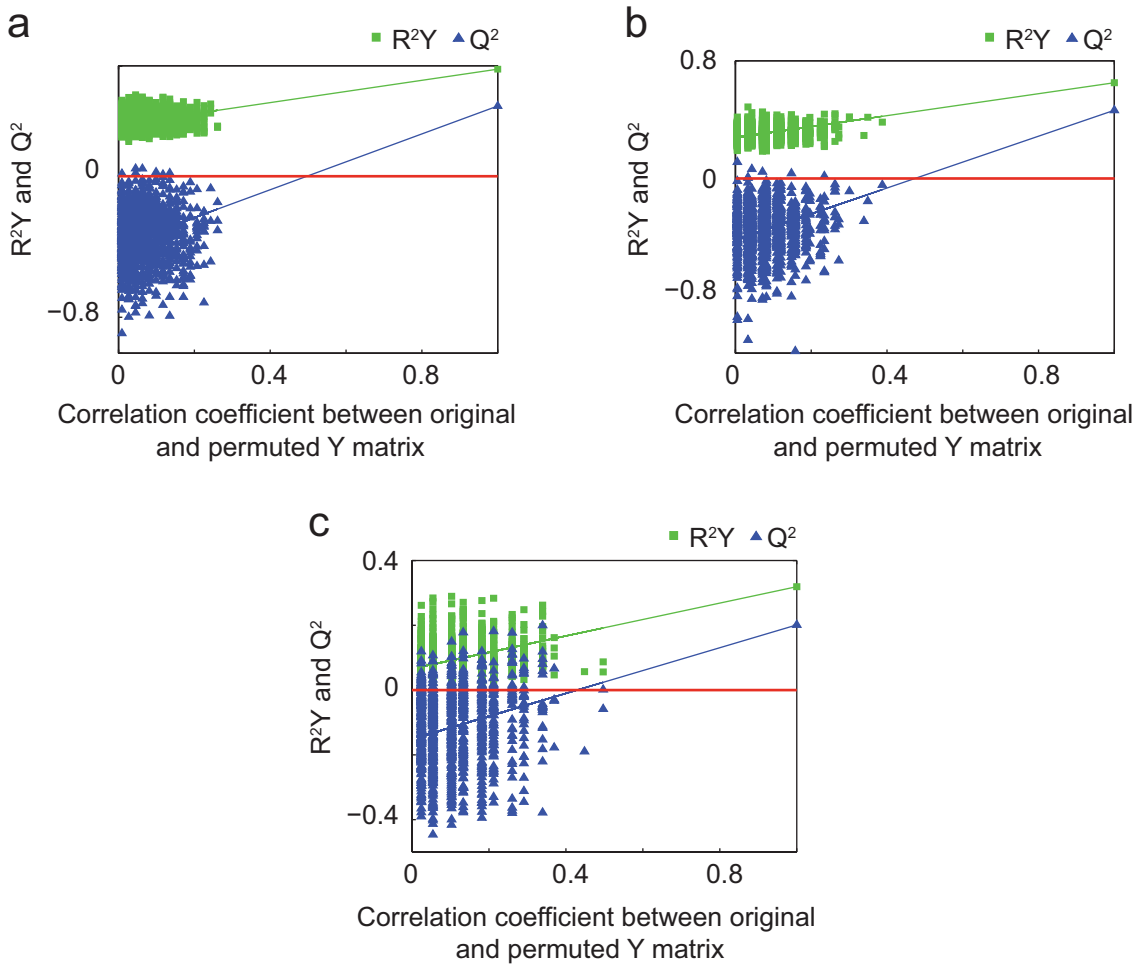
Supplementary Table 1: Metabolites identified from 800 MHz 1D and 2D NMR profiles of blood sera from patients of the TORAVA trial.

ID	Name	δ ^{1}H (ppm)	δ ^{13}C (ppm)	Multiplicity	Moieties	Observed
1	**1-methylhistidine**	7.04		s	***H4***	JRes,TOCSY
		7.76		s	***H2***	CPMG, JRes,TOCSY
2	**3-hydroxybutyratic acid**	1.19	24.7	d	γ***CH$_3$***	CPMG, JRes, TOCSY, HSQC
		2.30	49.1	q	half α***CH$_2$***	CPMG, JRes, TOCSY, HSQC
		2.40	49.1	q	half α***CH$_2$***	CPMG, JRes, TOCSY, HSQC
		4.13		m	β***CH***	JRes, TOCSY
3	**3-hydrophenylacetic acid**	6.86		m		CPMG, TOCSY
		7.24		t		CPMG, TOCSY
4	**Acetic acid**	1.91	25.7	s	***CH$_3$***	CPMG, JRes, TOCSY, HSQC
5	**Acetaoacetatic acid**	2.27		s	***CH$_3$***	CPMG, JRes
		3.43		s	α***CH$_2$***	CPMG, JRes
6	**Acetone**	2.22	32.9	s	***CH$_3$***	CPMG, JRes, TOCSY, HSQC
7	**Alanine**	1.46	19.3	d	β***CH$_3$***	CPMG, JRes, TOCSY, HSQC
		3.76		q	α***CH***	CPMG, JRes,TOCSY
8	**Albumin Lysyl**	2.89		t	ε***CH$_2$***	CPMG, TOCSY, JRes
		2.96	41.9	t	ε***CH$_2$***	CPMG, TOCSY, HSQC
		3.01	41.9	t	ε***CH$_2$***	CPMG, TOCSY, HSQC
9	**Aspartic Acid**	2.68		dd	half β***CH$_2$***	JRes, TOCSY
		2.80		dd	half β***CH$_2$***	JRes, TOCSY
		3.89		dd	α***CH***	JRes, TOCSY
10	**Betaine**	3.26	56.3	s	***CH$_3$***	CPMG, JRes, TOCSY, HSQC
11	**Cholesterol**	0.66	14.3	m	***C(18)H$_3$*** (in HDL)	CPMG, TOCSY
		0.70			***C(18)H$_3$*** (in VLDL)	CPMG, TOCSY
		0.82	25.1	m	***C(26)H$_3$*** and **C(27)H$_3$**	CPMG, TOCSY, HSQC
		0.91	21.1	d	***C(21)H$_3$***	CPMG, JRes, HSQC
		1.01		s	***C(19)H$_3$***	CPMG
		1.11		m		CPMG
12	**Choline**	3.19	56.8	s	N(***CH$_3$***)$_3$	CPMG, JRes, TOCSY
13	**Glycerophosphocholine**	3.22	56.5	s	N(***CH$_3$***)$_3$	CPMG, JRes, HSQC
		3.62		m	β***CH$_2$***	CPMG, JRes
		3.67		m	*N*α***CH$_2$***	CPMG, JRes
		4.29	64	m	α***CH$_2$***	JRes, HSQC
14	**O-Phosphocholine**	3.20	56.5	s	N(***CH$_3$***)$_3$	CPMG, TOCSY, JRes
		3.58		m	β***CH$_2$***	CPMG, JRes
15	**Citrate**	2.52		d	half ***CH$_2$***	CPMG, JRes, TOCSY
		2.67		d	half ***CH$_2$***	CPMG, JRes, TOCSY
16	**Creatine**	3.03	32.6	s	***CH$_3$***	CPMG, JRes, TOCSY, HSQC
		3.93		s	***CH$_2$***	CPMG, JRes, TOCSY, HSQC
17	**Creatine Phosphate**	3.03	32.6	s	***CH$_3$***	CPMG, JRes, TOCSY, HSQC
		3.93		s	***CH$_2$***	CPMG, JRes, TOCSY
18	**Creatinine**	3.04	32.6	s	***CH$_3$***	CPMG, JRes, TOCSY, HSQC
		4.05		s	***CH$_2$***	CPMG, JRes, TOCSY, HSQC
19	**Ethanol**	1.17		t	***CH$_3$***	CPMG, JRes, TOCSY
20	**Formate**	8.45		s	***CH***	CPMG, JRes, TOCSY
21	**β Galactose**	4.53		d	***H1***	JRes
22a	**α-Glucose**	3.42	72.2	q	***H4***	CPMG, JRes, TOCSY, HSQC
		3.53	74	dd	***H2***	CPMG, JRes, TOCSY, HSQC
		3.71	75.3	t	***H3***	CPMG, JRes, TOCSY, HSQC
		3.72	63.4	dd	half ***C(6)H$_2$***	CPMG, JRes, TOCSY, HSQC
		3.76	63.4	m	half ***C(6)H$_2$***	CPMG, JRes, TOCSY, HSQC
		3.83	74	ddd	***H5***	CPMG, JRes, TOCSY, HSQC
		3.84	63.2	m	half ***C(6)H$_2$***	CPMG, JRes, TOCSY, HSQC
		5.23	94.6	d	***H1***	CPMG, JRes, TOCSY, HSQC

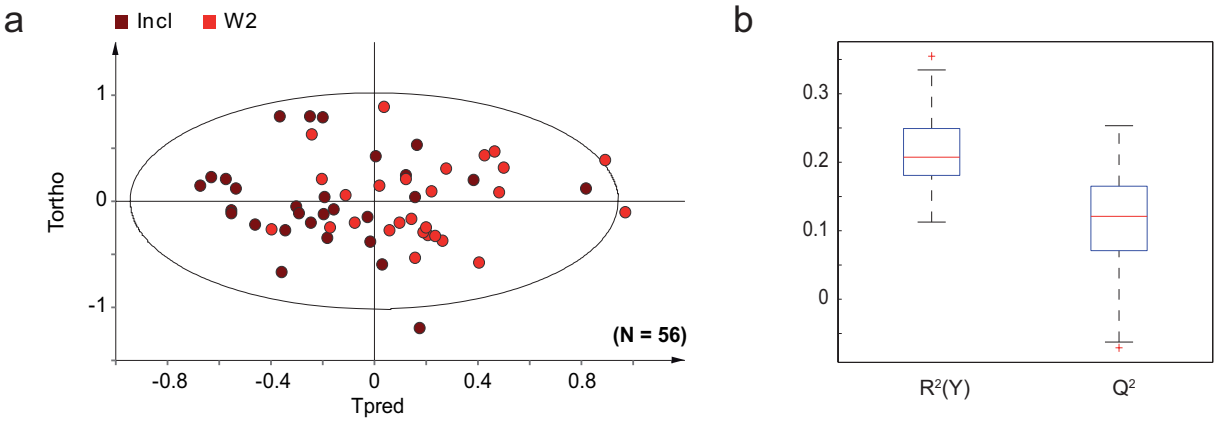
22b	**β Glucose**	3.24	76.8	dd	***H2***	CPMG, JRes, TOCSY, HSQC
		3.40	72.2	q	***H4***	CPMG, JRes, TOCSY, HSQC
		3.46	78.4	t	***H5***	CPMG, JRes, TOCSY, HSQC
		3.48	78.3	t	***H3***	CPMG, JRes, TOCSY, HSQC
		3.89	63.4	dd	half ***C(6)H$_2$***	CPMG, JRes, TOCSY, HSQC
		4.64	98.6	d	***H1***	CPMG, JRes, TOCSY, HSQC
23	**Glutamate**	2.04		m	half ***βCH$_2$***	CPMG, JRes, TOCSY
		2.11		m	half ***βCH$_2$***	CPMG, JRes, TOCSY
		2.34		m	half ***γCH$_2$***	CPMG, JRes, TOCSY
		3.72		m	***αCH***	JRes, TOCSY
24	**Glutamine**	2.11		m	half ***βCH$_2$***	CPMG, JRes, TOCSY
		2.46	33.9	m	half ***γCH$_2$***	JRes, TOCSY, HSQC
		3.67		t	***αCH$_2$***	JRes, TOCSY
25	**Glycerol**	3.56	65.9	dd	half ***CH$_2$***	CPMG, JRes, TOCSY, HSQC
		3.65	65.3	dd	half ***CH$_2$***	CPMG, JRes, TOCSY, HSQC
		3.88	74.2	dd	***C(2)H***	CPMG, JRes, TOCSY, HSQC
26	**Glycerol backbone**	4.06	64.2	m	***CH$_2$***OCOR	CPMG, JRes, TOCSY, HSQC
	of	4.25	64.2	m	***CH$_2$***OCOR	CPMG, JRes, TOCSY, HSQC
	PGLYs* and TAGs**	5.20		m	β***CH***OCOR	CPMG, JRes, TOCSY
27	**Glycine**	3.55	44.3	s	***CH$_2$***	CPMG, JRes, TOCSY, HSQC
28	**NAC*** 1**	2.04	24.8	s	NHCO***CH$_3$***	CPMG, JRes, TOCSY, HSQC
29	**NAC*** 2**	2.07	24.9	s	NHCO***CH$_3$***	CPMG, JRes, TOCSY, HSQC
30	**Histidine**	3.16		dd	half ***βCH$_2$***	CPMG, JRes, TOCSY
		3.98		dd	***αCH***	JRes
		7.03		s	***H4***	CPMG, JRes, TOCSY
		7.73		s	***H2***	CPMG, JRes, TOCSY
31	**Isoleucine**	0.93		t	***δCH$_3$***	CPMG, JRes, TOCSY
		1.00		d	***βCH$_3$***	CPMG, JRes, TOCSY
		1.28		m	α,γ ***CH$_2$***	JRes
		1.46		m	half γ**CH$_2$**	JRes, TOCSY
		1.95		m	β***CH***	JRes, TOCSY
32	**Isopropyl alcohol**	1.16		d	***CH$_3$***	CPMG, JRes, TOCSY
33	**Lactate**	1.32	25.7	d	***CH$_3$***	CPMG, JRes, TOCSY, HSQC
		4.10	71.1	q	***CH***	CPMG, JRes, TOCSY, HSQC
34	**Leucine**	0.94		d	***δCH$_3$***	CPMG, JRes, TOCSY
		0.95		d	***δCH$_3$***	CPMG, JRes, TOCSY
		1.71	42.7	m	***βCH$_2$***, ***γCH***	CPMG, JRes, TOCSY, HSQC
		3.69		dd	α***CH***	JRes, TOCSY
35	**Fatty acids (mainly LDL)**	0.84	16.5	t	***CH$_3$***$(CH_2)_n$	CPMG, JRes, TOCSY, HSQC
		1.23	34.5	m	$(***CH_2***)_n$	CPMG, JRes, TOCSY, HSQC
36	**Fatty acids (mainly VLDL)**	0.86	23.4	t	***CH$_3$***CH_2CH_2C=	CPMG, JRes, TOCSY, HSQC
		1.28	25.4	m	***CH$_2$***CH_2CH_2CO	CPMG, TOCSY, HSQC
		1.56	27.3	m	***CH$_2$***CH_2CO	CPMG, TOCSY, HSQC
37	**Fatty acids**	0.93	24.8	m	***CH$_3$***CH_2	CPMG, JRes, HSQC
		1.22	34.3	m	CH_3CH_2***CH$_2$***	CPMG,JRes, HSQC
		1.27	25.2	m	CH_3***CH$_2$*** $(CH_2)_n$	CPMG,JRes, HSQC
		1.30	25.4	m	***CH$_2$***	CPMG, JRes, TOCSY, HSQC
		1.32		m	***CH$_2$***CH_2CH_2CO	CPMG, JRes, TOCSY
		1.69	28.9	m	***CH$_2$***CH_2C=C	CPMG,JRes, HSQC
		2.0	29.5	m	$(CH_2)_n$***CH$_2$***CH=	CPMG, JRes, TOCSY, HSQC
		2.23	36.2	m	CH_2***CH$_2$***$COOC$	CPMG, JRes, TOCSY, HSQC
		2.74	28	m	$C=C$***CH$_2$***$C=C$	CPMG, JRes, TOCSY, HSQC
		5.23		m	***CH=CH***CH_2***CH=CH***	CPMG, TOCSY
		5.26	130.1	m	***CH=CH***CH_2***CH=CH***	CPMG, TOCSY, HSQC
		5.27		m	***=CH***CH_2CH_2	CPMG, TOCSY
		5.29	131.7	m	***CH=CH***CH_2***CH=CH***	CPMG, JRes, TOCSY, HSQC
		5.31		m	***=CH***CH_2CH_2	CPMG, TOCSY
		5.33		m	***=CH***CH_2CH_2	CPMG, JRes, TOCSY
38	**Lysine**	1.48		m	***γCH$_2$***	CPMG, JRes, TOCSY
		1.69		m	***δCH$_2$***	CPMG, TOCSY

		1.89		m	β***CH₂***	CPMG, JRes
		3.02		m	***εCH₂***	CPMG
39	**Mannose**	5.18		d	***CH***OH	CPMG, JRes, TOCSY
40	**Methanol**	3.34		s	O***CH₃***	CPMG, JRes, TOCSY
41	**Methionine**	2.13		s	S***CH₃***	JRes, TOCSY
42	**Phenylalanine**	3.25		dd	half β***CH₂***	JRes
		3.97		dd	α***CH***	JRes
		7.30		m	***H2, H6***	CPMG, TOCSY
		7.34		m	***H4***	CPMG, TOCSY
		7.40		m	***H3, H5***	CPMG, TOCSY
43	**Proline**	2.00		m	***γCH₂***	JRes
		2.06		m	half ***βCH₂***	JRes
		2.36		m	half ***βCH₂***	JRes
		3.35		m	half ***γCH₂***	CPMG, JRes
		4.12		m	α***CH***	JRes
44	**Pyruvate**	2.36		s	***CH₂***	CPMG, JRes, TOCSY
45	**Succinate**	2.40		s	***α,β CH₂***	Jres, TOCSY
46	**Threonine**	1.31		d	γ***CH₃***	JRes
		3.48		dd	α***CH***	JRes
		3.57		d	α***CH***	CPMG, JRes
		4.24		m	β***CH***	JRes
47	**Tyrosine**	6.87		d	***H3, H5***	CPMG, JRes, TOCSY
		7.18		d	***H2, H6***	CPMG, JRes, TOCSY
48	**Valine**	0.97	19.2	d	***CH₃***	CPMG, JRes, TOCSY, HSQC
		1.03	20.7	d	***CH₃***	CPMG, JRes, TOCSY, HSQC
		2.26		m	***βCH***	JRes, TOCSY
		3.60		d	***αCH***	CPMG, JRes, TOCSY
49	**Urea**	5.78		s	CO(***NH₂***)₂	CPMG, TOCSY

PGLYs*: Phosphoglycerides
TAGs**: Triacylglycerides
NAC***: N-acetyl-glycoprotein



Supplementary Figure 2: O-PLS Model validations by re-sampling 1000 times the model under the null hypothesis. a) OPLS model for the arm A, discriminating W0 versus W2. b) OPLS model for the arm A, discriminating W0 versus W5-6. c) OPLS model for the arm C, discriminating W0 versus W5-6.



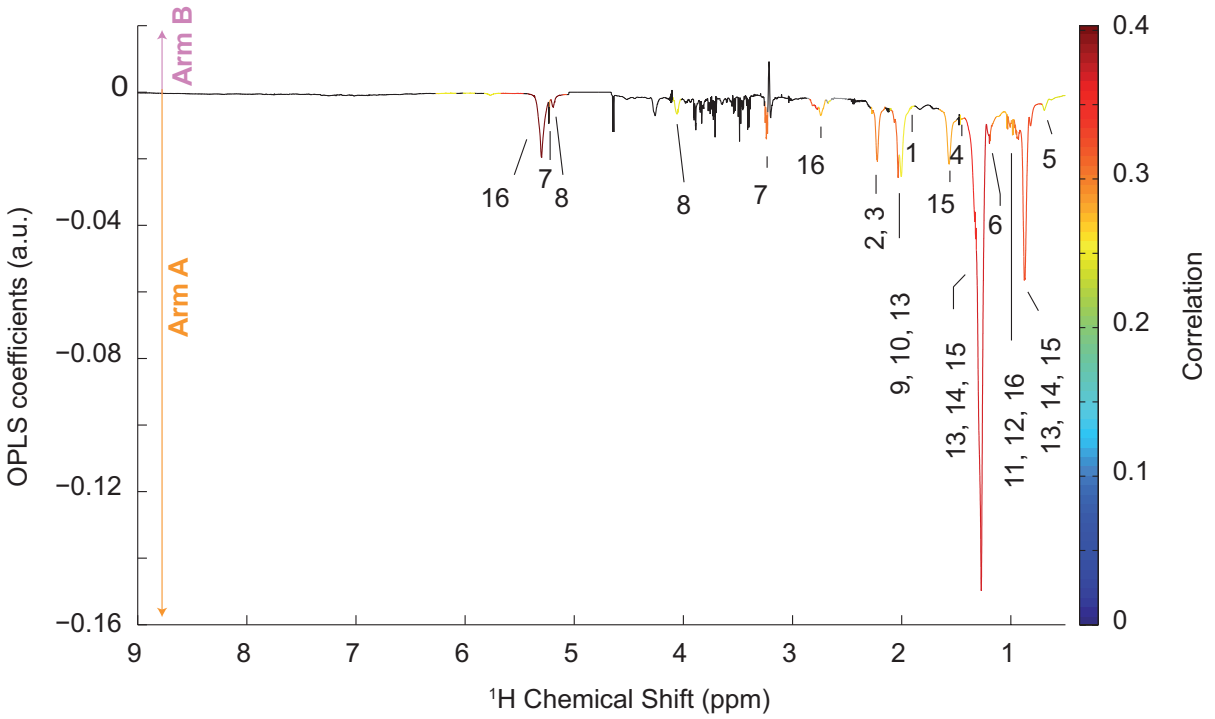
Supplementary Figure 3: Sensitivity analysis of O-PLS discrimination between W0 and W2 for the experimental arm A. a) Example of O-PLS model score plot (1+1 components) discriminating W0 vs. W2 for the experimental arm with N = 56, R^2X = 0.888, R^2Y =0.256 and Q^2 =0.163; b) Boxplots of R^2Y and Q^2 values obtained for 1,000 O-PLS models (1+1 components) build from random selection of sub-groups of 56 samples (W0: n=28; W2: n=28) from arm A. Boxplots describe the 25th and 75th percentiles (blue), the median (red), the wishers extend to the most extreme data points not considered outliers, and outliers are plotted individually.

Supplementary Table 2: Goodness-of-fit model parameters for PLS models discriminating the histoprognosis features of tumours, stratified by treatment (A, B & C) and collection time of serum samples (W0, W2 & W5-6).

		Treatment A (Temsirolimus + Bevacizumab)					Treatment B (Sunitinib)					Treatment C (Interferon-alpha + Bevacizumab)				
	PLS Model	**Nb. of samples**	**Nb. of components**	**R^2X**	**R^2Y**	**Q^2**	**Nb. of samples**	**Nb. of components**	**R^2X**	**R^2Y**	**Q^2**	**Nb. of samples**	**Nb. of components**	**R^2X**	**R^2Y**	**Q^2**
W0	**Tumor Type**	56	2	0.915	0.045	-0.029	26	2	0.867	0.105	-0.127	/	/	/	/	/
	Grade Furhman (I & II vs. III & IV)	43	2	0.913	0.086	-0.113	24	2	0.913	0.184	-0.132	27	2	0.905	0.0579	-0.196
	Interval bewteen diagnosis & metastasis (≤ 12 months vs. > 12 months)	56	2	0.922	0.024	-0.067	25	2	0.91	0.353	0.161	30	2	0.85	0.261	-0.21
	PS (0 or 1 vs. 2)	56	2	0.926	0.137	-0.116	/	/	/	/	/	/	/	/	/	/
	MSKKC Classification															
	Poor vs. Intermediate prognosis	35	2	0.91	0.044	-0.113	15	2	0.888	0.29	-0.21	15	2	0.778	0.523	-0.21
	Poor vs. Favorable prognosis	26	2	0.94	0.092	-0.091	11	2	0.789	0.557	0.184	15	2	0.883	0.423	0.181
	Intermediate vs. Favorable prognosis	43	2	0.91	0.045	-0.162	24	2	0.694	0.102	-0.21	22	2	0.871	0.26	0.0153
W2	**Tumor Type**	55	2	0.924	0.053	-0.079	22	2	0.868	0.149	-0.2	/	/	/	/	/
	Grade Furhman (I & II vs. III & IV)	42	2	0.929	0.07	-0.128	20	2	0.907	0.0923	-0.21	22	2	0.939	0.104	-0.145
	Interval bewteen diagnosis & metastasis (≤ 12 months vs. > 12 months)	55	2	0.93	0.057	-0.023	21	2	0.769	0.314	-0.21	25	2	0.891	0.224	-0.0191
	PS (0 or 1 vs. 2)	55	2	0.9	0.041	-0.045	/	/	/	/	/	/	/	/	/	/
	MSKKC Classification															
	Poor vs. Intermediate prognosis	35	2	0.895	0.117	-0.088	14	2	0.909	0.283	-0.21	11	2	0.96	0.319	-0.0809
	Poor vs. Favorable prognosis	22	2	0.902	0.182	-0.056	7	2	0.934	0.945	0.862	13	2	0.955	0.241	-0.107
	Intermediate vs. Favorable prognosis	45	2	0.934	0.18	0.049	19	2	0.888	0.336	-0.0722	18	2	0.925	0.149	0.119
W5-6	**Tumor Type**	49	2	0.872	0.163	-0.126	20	2	0.92	0.145	-0.165	/	/	/	/	/
	Grade Furhman (I & II vs. III & IV)	40	2	0.942	0.098	-0.034	19	2	0.916	0.149	-0.167	20	2	0.945	0.151	-0.166
	Interval bewteen diagnosis & metastasis (≤ 12 months vs. > 12 months)	49	2	0.859	0.158	-0.207	20	2	0.92	0.198	-0.147	22	2	0.898	0.374	-0.0651
	PS (0 or 1 vs. 2)	49	2	0.933	0.064	-0.11	/	/	/	/	/	/	/	/	/	/
	MSKKC Classification															
	Poor vs. Intermediate prognosis	36	2	0.871	0.149	-0.075	9	2	0.947	0.367	-0.145	10	2	0.928	0.7	0.35
	Poor vs. Favorable prognosis	20	2	0.91	0.19	-0.131	10	2	0.881	0.526	-0.108	19	2	0.945	0.235	0.0112
	Intermediate vs. Favorable prognosis	38	2	0.942	0.057	-0.098	17	2	0.842	0.247	-0.21	15	2	0.94	0.353	0.0749

Supplementary Table 3: Goodness-of-fit model parameters for O-PLS models discriminating the experimental arm and the two standard therapies according to the collection time of serum samples (W0, W2 & W5-6).

Collection Time	Model	Sample Number	Orthogonal Component	R^2X	R^2Y	Q^2	CV-ANOVA *p*-value
W0	**A vs B**	82	1	0.916	0.048	-0.018	1
	A vs C	86	1	0.791	0.078	-0.145	1
	B vs C	56	1	0.896	0.076	0.020	0.897
W2	**A vs B**	77	2	0.951	0.26	0.144	0.081
	A vs C	80	3	0.959	0.35	0.186	0.056
	B vs C	47	1	0.914	0.206	0.173	0.086
W5-6	**A vs B**	69	2	0.957	0.355	0.188	0.038
	A vs C	71	2	0.956	0.28	0.128	0.171
	B vs C	42	2	0.954	0.453	0.252	0.097



Supplementary Figure 4: O-PLS loadings plot after univariate analysis at W5-6 between arm A and B. Statistically significant signals correspond to coloured spectral regions. Highlighted candidate markers are: 1) Acetate, 2) Acetoacetate, 3) Acetone, 4) Alanine, 5) Cholesterol, 6) 3-hydroxybutyrate, 7) Glucose, 8) Glycerol backbone of PGLYs and TAGs, 9) N-acetylglycoprotein (NAC1), 10) NAC2, 11) Isoleucine, 12) Leucine, 13) Fatty acids (mainly LDL), 14) Fatty acids (mainly VLDL), 15) Fatty acids and 16) Valine. PGLY: Phosphoglycerides; TAGs: Triglycerides.