A distinct plasma lipid signature associated with poor prognosis in castration-resistant prostate cancer

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Short title

Prognostic lipid signature in metastatic prostate cancer

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Keywords

Lipids, prostate cancer, castration-resistant, biomarker, prognosis

Abbreviations

ALP, Alkaline phosphatase
BMI, body mass index
CI, confidence interval
CRPC, castration-resistant prostate cancer
HR, hazard ratio
LC-MS/MS, liquid chromatography and electrospray ionisation-tandem mass spectrometry
N, number of patients
P, p-value
PSA, prostate-specific antigen

Novelty and impact

The association of circulating lipids with the clinical outcome of metastatic castration-resistant prostate cancer (CRPC) is unknown. We performed lipidomic analysis on plasma from CRPC patients and identified a three-lipid signature associated with overall survival – elevated plasma levels of ceramide d18:1/24:1, sphingomyelin d18:2/16:0, and phosphatidylcholine 16:0/16:0 were associated with worse outcome. The signature was independent of clinicopathological factors and metabolic characteristics, and was validated in an independent cohort.

Abstract

Lipids are known to influence tumour growth, inflammation, and chemoresistance. However, the association of circulating lipids with the clinical outcome of metastatic castration-resistant prostate cancer (CRPC) is unknown. We investigated associations between the plasma lipidome and clinical outcome in CRPC. Lipidomic profiling by liquid chromatography-tandem mass spectrometry was performed on plasma samples from a Phase 1 discovery cohort of 96 CRPC patients. Results were validated in an independent Phase 2 cohort of 63 CRPC patients. Unsupervised analysis of lipidomic profiles (323 lipid species) classified the Phase 1 cohort into two patient subgroups with significant survival differences (HR 2.31, 95% CI 1.44-3.68, *P*=0.0005). The levels of 46 lipids were individually prognostic, and were predominantly sphingolipids with higher levels associated with poor prognosis. A prognostic three-lipid signature was derived (ceramide d18:1/24:1, sphingomyelin d18:2/16:0, phosphatidylcholine 16:0/16:0), and was also associated with shorter survival in the Phase 2 cohort (HR 4.8, 95% CI 2.06-11.1, *P*=0.0003). The signature was an independent prognostic factor when modelled with clinicopathological factors or metabolic characteristics. The association of plasma lipids with CRPC prognosis suggests a possible role of these lipids in disease progression. Further research is required to determine if therapeutic modulation of the levels of these lipids by targeting their metabolic pathways may improve patient outcome.

Introduction

Although prostate cancer is initially sensitive to hormonal manipulation, resistance to androgen deprivaiton therapy ultimately occurs with the development of metastatic castration-resistant prostate cancer (CRPC). Despite a range of new therapeutics for metastatic CRPC,[1] patients eventually develop resistance to these treatments. Thus there is still a need for new biomarkers and treatment strategies to improve patient outcome.

The importance of lipid metabolism in prostate cancer is reflected by various epidemiological and molecular evidence. Higher incidence of aggressive prostate cancer and prostate cancer-specific mortality are observed in obese men.[2] Prostate cancer cells display increased lipid lipogenesis and lipolysis, and altered metabolism of cholesterol and phospholipids.[3]

Lipids contribute to cancer growth as energy substrates, cell membrane constituents, and signalling molecules in the regulation of cell cycle, apoptosis, angiogenesis, and inflammation.[4, 5] Lipids may also influence chemoresponse, where a higher degree of lipid saturation in membranes was shown to promote the chemoresistance of prostate cancer cells.[6]

However, the role of circulating lipids on prostate cancer biology is unclear, despite the presence of ~500 distinct lipid species from six main lipid classes in human plasma.[7] These circulating lipids may influence the behaviour of cancer and immune cells, and may include lipids released by cancer cells. Studies of circulating lipids in prostate cancer have been mainly focused on a few lipid species (e.g. cholesterol, triacylglycerols), or the fatty acid profile of a lipid class (e.g. omega-3 fatty acid in phospholipids) in localised prostate cancer.[8, 9] There are no studies of circulating lipids in metastatic CRPC.

The aim of this study was to investigate whether comprehensive lipidomic profiling can identify circulating lipids that are associated with overall survival and response to docetaxel chemotherapy in men with CRPC in a Phase 1 discovery cohort, and to corroborate these findings in an independent Phase 2 validation cohort.

Patients and Methods

Patients and plasma collection

Plasma samples were acquired from men with CRPC commencing docetaxel (75 mg/m^2) and prednisone (5 mg twice daily) at seven hospitals in New South Wales, Australia between 2006 and 2015 (Appendix A). Phase 1 and Phase 2 cohorts were independently defined (Appendix A). All participants provided written informed consent (human research ethics approval number X14-0406; Australian-New Zealand Clinical Trials Registry ACTRN12607000077460).

Plasma samples were non-fasting, and obtained before the first cycle of docetaxel (baseline), and three weeks later before the second cycle (post-docetaxel). The plasma samples were collected according to a standardised blood collection protocol for all sites. Blood samples were collected in BD Vacutainer tubes containing K_2EDTA, and processed by centrifugation at 3000 g for 5 minutes at room temperature. The plasma was removed with a disposable transfer pipette, aliquoted into cryovials, and stored at -80^{o}C.

Chemoresponse was based on serum prostate-specific antigen (PSA) response as defined by the Prostate Cancer Clinical Trials Working Group (Appendix A). Overall survival was defined as the time from the first cycle of docetaxel to the date of death or last known to be alive.

Lipidomic profiling

Lipidomic profiling of plasma samples was performed by liquid chromatography and electrospray ionisation-tandem mass spectrometry (LC-MS/MS) as reported by Weir and colleagues[10] with minor modifications (Appendix B). Plasma samples were subjected to one freeze-thaw when aliquoted for lipidomic analysis. Lipid levels (pmol/ml) were normalised by the Probabilistic Quotient Normalisation method[11] (Appendix C). Final normalised levels were in logarithm-2 scale.

Statistical analysis

Statistical analyses were done with R software version 3.2.1 or IBM SPSS version 23.0.0.0. Heatmap of lipid levels was created using HeatMapViewer in GenePattern version 3.2.3.

Latent class analysis was performed on normalised baseline lipid levels of the Phase 1 cohort, as quartiles, to identify patient subgroups (latent classes) that are associated with outcome (R package "poLCA" version 1.4.1). The minimum Bayesian Information criterion was used to determine the most parsimonious number of latent classes. Lipid species with significantly different levels between the patient subgroups were identified by multiple t-test (R package "multtest" version 2.24.0). The association of these lipid species with overall survival was determined by univariable Cox regression (R package "survival" version 2.38-1).

A lipid signature was derived from the baseline levels of those lipids associated with overall survival, by multivariable logistic regression with backward stepwise variable selection and 3-fold cross-validation repeated 200 times (R package "caret" version 6.0-52).

Results

Patient cohorts

The study schema is displayed in Figure 1. The Phase 1 discovery cohort comprised 96 patients, whereas the Phase 2 validation cohort comprised 63 patients (Appendix A). The baseline characteristics of both cohorts were similar, except that fewer patients of the Phase 2 cohort had soft tissue metastases and PSA responses to docetaxel (Table 1). The median overall survival time for the Phase 1 and Phase 2 cohorts was 17.8 months (95% CI 14.6-21.1) and 16.3 months (95% CI 11.5-21.0) respectively.

Phase 1 cohort: association of plasma lipidomic profiles with survival

LC-MS/MS lipidomic profiling of plasma samples from the Phase 1 cohort quantified 323 lipid species comprising 22 lipid classes/subclasses from four main lipid categories – sphingolipids, glycerophospholipids, glycerolipids, and sterols (Appendix D) .

Latent class analysis of the baseline levels of these 323 lipids optimally classified the 96 patients into two subgroups: Profile-1 and Profile-2. Interestingly, Profile-2 patients had a significantly shorter overall survival than Profile-1 patients (median overall survival 13.7 versus 21.7 months, P=0.0003) (Figure 2A).

The baseline levels of 164 lipids were significantly different between Profile-1 and Profile-2 patients, of which 46 were individually significantly associated with overall survival ($P \leq 0.05$, False Discovery Rate [FDR]≤10%, Appendix Table E1 & Figure E1). Half of these lipids were sphingolipids, where their baseline levels were higher in Profile-2 patients compared to Profile-1. The total baseline levels of each of the sphingolipid subclasses were also significantly different between the two patient subgroups ($P \leq 0.007$, Appendix Table E2).

A prognostic model was derived from the 46 significant lipids above, consisting of three lipid species – ceramide(d18:1/24:1), sphingomyelin(d18:2/16:0) and phosphatidylcholine(16:0/16:0) (Figure 2B, Appendix Table F1). Patients with this three-lipid signature have a shorter overall survival time (11.7 versus 21.7 months, P=0.00001; Figure 2C).

Phase 1 cohort: comparison of lipid signature to clinicopathological factors

The three-lipid signature, and alkaline phosphatase and haemoglobin levels were independent predictors of overall survival ($P \leq 0.05$), when the signature was modeled with clinicopathological factors in multivariable Cox regression (Table 2). Patients with the three-lipid signature have significantly higher levels of alkaline phosphatase and PSA, and significantly lower levels of haemoglobin, than those without the signature ($P \leq 0.006$, Appendix Table F2).

The survival difference between the risk groups defined by the three-lipid signature (11.7 versus 21.7 months, P=0.00001; HR 2.94, 95% CI 1.80-4.81) were significantly larger than those defined by Halabi's prognostic model for CRPC (15.5 versus 24.2 months, P=0.05; HR 1.68, 95% CI 0.99-2.84), and a clinicopathological model derived from multivariable Cox regression (12.5 versus 21.4 months, P =0.002; HR 2.00, 95% CI 1.27-3.18) (Appendix Table F3 & Figure F1).

The area under the curve (AUC) of receiver operating characteristic (ROC) analysis of 12 months survival for the clinicopathological model (AUC 0.70, 95% CI 0.58-0.82, P=0.003) was slightly enhanced by the addition of the three-lipid signature (AUC 0.75, 95% CI 0.64-0.86, P=0.0002). The combination was higher than that of Halabi's prognostic model (AUC 0.72, 95% CI 0.59-0.84, P=0.001) and the lipid signature alone (AUC 0.67, 95% CI 0.54-0.81, P=0.009) (Appendix Table F5).

The association of the three-lipid signature with overall survival was not confounded by metabolic factors related to metabolic diseases, as the three-lipid signature was the only independent factor associated with overall survival when modelled with body mass index (BMI), cholesterol levels, and triacylglycerol levels (Appendix Table F6).

The rate of diabetes, and statin/metformin usage was not significantly correlated with the three-lipid signature, nor were they predictors of overall survival, where the three-lipid signature remained an independent prognostic factor when modelled with these factors (Appendix Table F7 & F8).

Phase 2 cohort: validation of prognostic lipids and lipid signature

Of the 46 lipids that were associated with overall survival in the Phase 1 cohort, the baseline levels of 19 including the three lipids comprising the lipid signature, were significantly associated with overall survival in the Phase 2 cohort ($P \leq 0.03$, Table 3, Figure 2D's heatmap). The majority of these were sphingolipids.

Eleven of the 63 patients of the Phase 2 cohort expressed the three-lipid signature, and their median overall survival time was significantly shorter than those without the three-lipid signature (11.3 versus 21.4 months, $P=0.00007$; HR 4.78, 95% CI 2.06-11.1, $P=0.0003$; Figure 2D). The survival difference between the risk groups defined by the three-lipid signature was larger than those defined by Halabi's prognostic model (13.9 versus 20.6 months, $P=0.095$; HR 1.82, 95% CI 0.89-3.7), and the clinicopathological model described above (11.5 versus 20.6 months, $P=0.03$; HR 2.53, 95% CI 1.05-6.10) (Appendix Table G1 & Figure G1).

The clinicopathological model predicting 12 months survival (AUC 0.70, 95% CI 0.54-0.87, $P=0.03$) was slightly enhanced by the addition of the three-lipid signature (AUC 0.73, 95% CI 0.57-0.89, $P=0.01$), and was comparable to that of Halabi's prognostic model (AUC 0.74, 95% CI 0.60-0.88, $P=0.01$) (Appendix Table G2). However, the three-lipid signature alone had the highest AUC of 0.79 (95% CI 0.64-0.94, $P=0.002$).

The three-lipid signature was an independent prognostic factor when analysed together with clinicopathological factors in multivariable analysis (Table 2). A higher proportion of patients with the three-lipid signature had significantly lower levels of haemoglobin ($P=0.001$), but there were no significant differences for the other clinicopathological factors (Appendix Table G3).

The three-lipid signature also remained an independent prognostic factor when modelled with BMI, plasma cholesterol or total triacylglycerol levels (Appendix Table G4). There was no association between the lipid signature and metformin/statin use, or diabetic status, where the lipid signature remained an independent prognostic factor when modelled with these factors (Appendix Tables G5-G9).

Assessment of lipidomic profiles with chemoresponse

PSA response to docetaxel and the patient subgroups identified by latent class analysis in the Phase 1 cohort were both independent factors of survival according to bivariable Cox regression (Appendix Table H1). The proportion of PSA responses was not significantly different between Profile-1 and Profile-2 subgroups (Appendix Table H2). The baseline levels or post-docetaxel change in the levels of individual lipid species were not reliably associated with PSA response to docetaxel (Appendix Tables H3 & H4).

Discussion

In this study, we discovered that the plasma lipidomic profile of CRPC patients is associated with overall survival, independent of their PSA response to docetaxel. We identified and independently validated 19 lipid species associated with overall survival. We also developed and validated a three-lipid signature that was associated with substantially shorter overall survival.

Prognostic biomarkers in CRPC (e.g. Halabi nomogram, circulating tumour cells) are normally of academic interest with little clinical utility outside of clinical trials stratification. The three-lipid signature was not associated with response to docetaxel, and thus should not be used to decide if patients should undergo or continue with chemotherapy. Instead, the three-lipid signature may have clinical utility as a biomarker to identify CRPC patients in whom therapeutic modulation of their lipid profile in combination with standard of care CRPC treatments may influence their survival. This application of the signature is dependent on whether the lipids associated with poor outcome are influencing the growth of the cancer, and if changing a patient's lipid profile with therapeutic agents can improve his prognosis. Of particular interest as a therapeutic target is the observation that the majority of lipid species associated with prognosis belong to the sphingolipid class.

Sphingolipids are highly bioactive, and participate in various cellular signalling pathways regulating apoptosis, proliferation, and immune cell trafficking.[5] Intracellularly synthesised sphingolipids are considered to be tumour suppressors, as chemotherapy induced the production of intracellular ceramides which caused apoptosis.[12] In contrast, we found that high levels of circulating sphingolipids was associated with poor prognosis in CRPC. This may relate to the role of sphingolipids being context-dependent, where circulating sphingolipids may have a distinct role from those synthesised intracellularly in the cancer cell.

The role of sphingolipids in inflammatory and metabolic diseases may provide mechanistic insights into the association of high circulating levels with poor prognosis in CRPC. The pro-inflammatory effects of sphingolipids through regulation of immune cell trafficking and differentiation, and cytokine production, may indirectly enhance tumour growth.[13] Promotion of vascular permeability and endothelial cell dysfunction by ceramides may also enhance tumour metastasis.[13] Furthermore, hyperinsulinemia arising from ceramide-induced insulin-resistance may activate insulin/insulin-like-growth-factor-1-regulated mitogenic and anti-apoptotic pathways in cancer cells.[14, 15]

The lipid profile of poor prognosis in our study is similar to that of cardiovascular disease and Type 2 diabetes in terms of elevation of circulating ceramides, and/or free cholesterol.[16-19] Statin therapy reduces the plasma levels of ceramides, sphingomyelin, and cholesterol in individuals with cardiovascular disease or metabolic syndrome,[20-22] suggesting that such therapy could convert the high risk lipid profile of CRPC patients to a low risk lipid profile and thereby improve their prognosis. A meta-analysis of observational studies on statin usage in primary prostate cancer showed that statin usage was associated with a reduced rate of relapse and prostate cancer-specific mortality.[23] However, data is limited and conflicting for statin usage in CRPC, with one study reporting the lack of survival benefits of statin on CRPC patients treated with abiraterone.[24] The beneficial effects of statin on CRPC survival may be dependent on the lipid profile of the patients, and thus our lipid signature would be useful in identifying such patients.

Other medications that may modulate our high risk lipid profile include the insulin sensitiser pioglitazone, which lowered circulating ceramide levels in individuals with metabolic syndrome;[25] and inhibitors that target sphingolipid metabolism, such as fingolimod which is used as an immunosuppressive in multiple sclerosis.[26]

A limitation of this study is the inability to identify the source of the circulating lipids. The lipid classes identified in our study are predominantly transported in the circulation in lipoprotein pools.[27] An intriguing possibility is that extracellular vesicles from malignant cells could contribute to the lipid profile. Exosomes secreted by tumour cells have significant biological activity such as promotion of metastasis and tumour growth.[28] Interestingly, exosomes from prostate cancer cells were found to be enriched in sphingolipids and cholesterol.[29]

Our study demonstrated that plasma lipidomic profiles were not associated with response to docetaxel. Some of the patients of both cohorts were treated with the new therapeutic agents (abiraterone, enzalutamide, and cabazitaxel) after docetaxel. However, there were insufficient patient numbers to determine if the plasma lipidomic profiles were associated with response to these new treatments.

In summary, we have identified and validated a novel three-lipid signature associated with poor prognosis in CRPC. Further research is required to determine if therapeutic modulation of lipid levels associated with poor prognosis can improve patient outcome.

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Figure legends

Figure 1.
Study schema and data analysis strategy

Figure 2.
Survival curves, lipid signature, and heatmaps of prognostic baseline plasma lipid levels. **(A)** Survival curves of Phase 1 discovery cohort classified by latent class analysis of baseline lipidomic profile; **(B)** Three-lipid signature of normalised baseline lipid levels; **(C)** Survival curves of Phase 1 discovery cohort classified by the three-lipid signature, and heatmap of 19 prognostic lipids validated in Phase 2; **(D)** Survival curves of Phase 2 validation cohort classified by the three-lipid signature, and heatmap of the 19 validated prognostic lipids.

Table 1. Patient characteristics of Phase 1 and 2 cohorts.

	Phase 1 cohort		Phase 2 cohort	
	N (%)	**Median (range)**	**N (%)**	**Median (range)**
Age (years)	96 (100)	68.9 (46.2-86.8)	63 (100)	71.6 (40.1-88.6)
Body mass index	73 (76)	27 (18-42)	49 (78)	28.1 (22-44)
Follow-up (months)	96 (100)	15.9 (1.5-63.5)	63 (100)	13.3 (0.7-39)
Status at censoring				
Alive	18 (19)		29 (46)	
Dead	78 (81)		34 (54)	
PSA response				
Partial response	50 (52)		26 (41)	
Stable disease	28 (29)		16 (25)	
Progressive disease	17 (18)		13 (21)	
Gleason at diagnosis				
≤6	4 (4.2)		7 (11)	
7	21 (22)		13 (20)	
8-10	52 (54)		24 (38)	
Serum PSA baseline (µg/l)	96 (100)	133 (3-10054)	60 (95)	94.9 (1.1-4035)
Haemoglobin baseline (g/l)	96 (100)	124.5 (67-168)	60 (95)	126.5 (81-164)
ALP baseline (U/l)	96 (100)	137 (15-2962)	58 (92)	123 (16-1426)
ECOG performance				
0	11 (12)		8 (13)	
1	36 (38)		12 (19)	
2	7 (7.3)		5 (7.9)	
Albumin baseline (g/l)	54 (56)	42 (32-50)	25 (40)	43 (38-48)
Opioid analgesic use				
User	21 (22)		7 (11)	
Unknown	43 (45)		38 (60)	
Metastasis				
None	3 (3.1)		1 (1.6)	
Bone only	53 (55)		41 (65)	
Soft tissue	39 (41)		20 (32)	
Diabetes				
Diagnosed	12 (13)		11 (18)	
Unknown	3 (3.1)		3 (4.8)	
Concurrent treatments				
Statin	25 (26)		24 (38)	
Fibrate	1 (1.0)		0	
Metformin	8 (8.3)		8 (13)	
Unknown	3 (3.1)		3 (4.8)	
Subsequent treatments				
Abiraterone	47 (49)		35 (55.6)	
Enzalutamide	4 (4.2)		21 (33)	
Cabazitaxel	36 (38)		18 (29)	
Mitoxantrone	20 (21)		6 (9.5)	
Docetaxel	4 (4.2)		0	
Strontium	2 (2.1)		0	
Other*	8 (8.3)		2 (3.2)	
Unknown	4 (4.2)		3(4.8)	

*carboplatin/etoposide, vinorelbine, c-met inhibitor, cyclophosphamide, paclitaxel, cabozantinib
N, number of participants; PSA, prostate-specific antigen; ALP, alkaline phosphatase

Table 2. Results of Cox regression assessing the relationships between the three-lipid signature, clinicopathological factors, and overall survival in the Phase 1 and 2 cohorts.

Cohort	Variables [a]	cases	events	Univariable analysis		Multivariable analysis [b]	
				Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Phase 1	Three-lipid signature (absent vs present)	96	78	2.94 (1.80-4.81)	$2x10^{-5}$	2.90 (1.72-4.89)	$7x10^{-5}$
	Alkaline phosphatase	96	78	1.00 (1.00-1.00)	0.009	1.00 (1.00-1.00)	0.01
	Haemoglobin	96	78	0.99 (0.98-1.00)	0.01	0.99 (0.98-1.00)	0.047
	Serum PSA	96	78	1.00 (1.00-1.00)	0.57	1.00 (1.00-1.00)	0.65
	Metastasis site (bone only/none vs soft tissue)[c]	95	77	1.09 (0.69-1.72)	0.71	1.22 (0.77-1.94)	0.41
Phase 2	Three-lipid signature (absent vs present)	61	34	4.78 (2.06-11.1)	0.0003	3.63 (1.24-10.6)	0.02
	Alkaline phosphatase	56	33	1.0 (1.00-1.00)	0.19	1.00 (1.00-1.00)	0.83
	Haemoglobin	58	33	0.97 (0.94-0.99)	0.008	0-98 (0.95-1.01)	0.11
	Serum PSA	58	33	1.00 (1.00-1.00)	0.05	-	-
	Metastasis site (bone only/none vs soft tissue)[c]	61	34	0.84 (0.37-1.87)	0.66	-	-

[a] analysed as continuous variable unless stated otherwise
[b] multivariable analysis for Phase 2 cohort only performed on variables significant in multivariable analysis of Phase 1 cohort
[c] visceral and nodal metastases were not distinguished in our database

Table 3. Hazard ratio of baseline plasma levels of 19 validated prognostic lipids, analysed as continuous variables in univariable Cox regression.

Lipid	Phase 1 cohort			Phase 2 cohort		
	Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
Cer(d18:1/16:0)	2.20	1.34-3.60	0.003	10.20	3.19-32.6	0.0003
Cer(d18:1/18:0)	1.74	1.24-2.45	0.002	4.45	2.16-9.19	0.00006
Cer(d18:1/20:0)	1.86	1.23-2.83	0.005	5.24	2.09-13.2	0.0005
Cer(d18:1/24:1)	2.56	1.51-4.35	0.0007	2.90	1.13-7.46	0.02
HexCer(d18:1/16:0)	2.37	1.47-3.83	0.0003	2.75	1.26-6.00	0.01
GM3(d18:1/16:0)	2.94	1.70-5.05	0.0001	5.60	1.73-18.1	0.004
GM3(d18:1/20:0)	1.79	1.16-2.76	0.009	3.29	1.19-9.08	0.02
SM(d18:1/16:0)	3.51	1.49-8.28	0.004	9.99	2.51-39.7	0.0009
SM(d18:2/16:0)	4.82	2.04-11.4	0.0004	4.36	1.11-17.2	0.03
SM(36:1)	2.29	1.28-4.10	0.007	4.25	1.74-10.4	0.002
SM(d18:2/18:0)	2.11	1.20-3.70	0.01	3.94	1.55-10.1	0.004
SM(d18:2/20:0)	2.23	1.18-4.19	0.01	3.20	1.13-9.10	0.02
PC(16:0/16:0)	4.72	1.93-11.6	0.0007	12.51	2.68-58.3	0.0007
PC(38:2)	2.60	1.30-5.19	0.006	3.40	1.56-7.38	0.003
PC(P-16:0/20:4)	2.06	1.09-3.88	0.02	4.99	1.53-16.3	0.006
Free cholesterol	4.06	1.33-12.4	0.01	7.86	1.40-44.1	0.02
PC(38:6)	0.52	0.31-0.88	0.01	0.21	0.07-0.61	0.005
PC(14:0_20:4)	0.64	0.45-0.92	0.02	0.45	0.23-0.88	0.02
PC 40:8	0.41	0.23-0.75	0.005	0.21	0.07-0.69	0.01

Cer, ceramide; HexCer, monohexosylceramide; GM3, GM3 ganglioside; SM, sphingomyelin; PC, phosphatidylcholine; PC(P), alkenylphosphatidylcholine

FIGURE 1

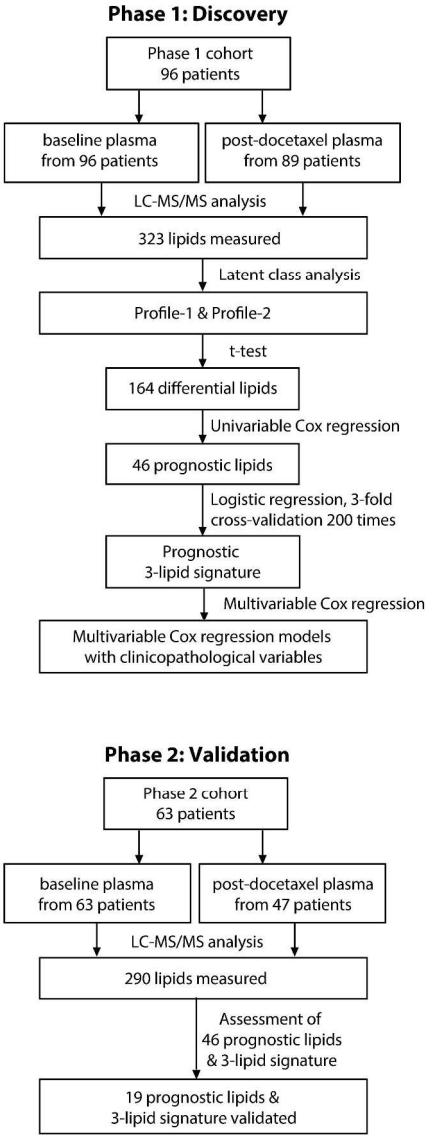
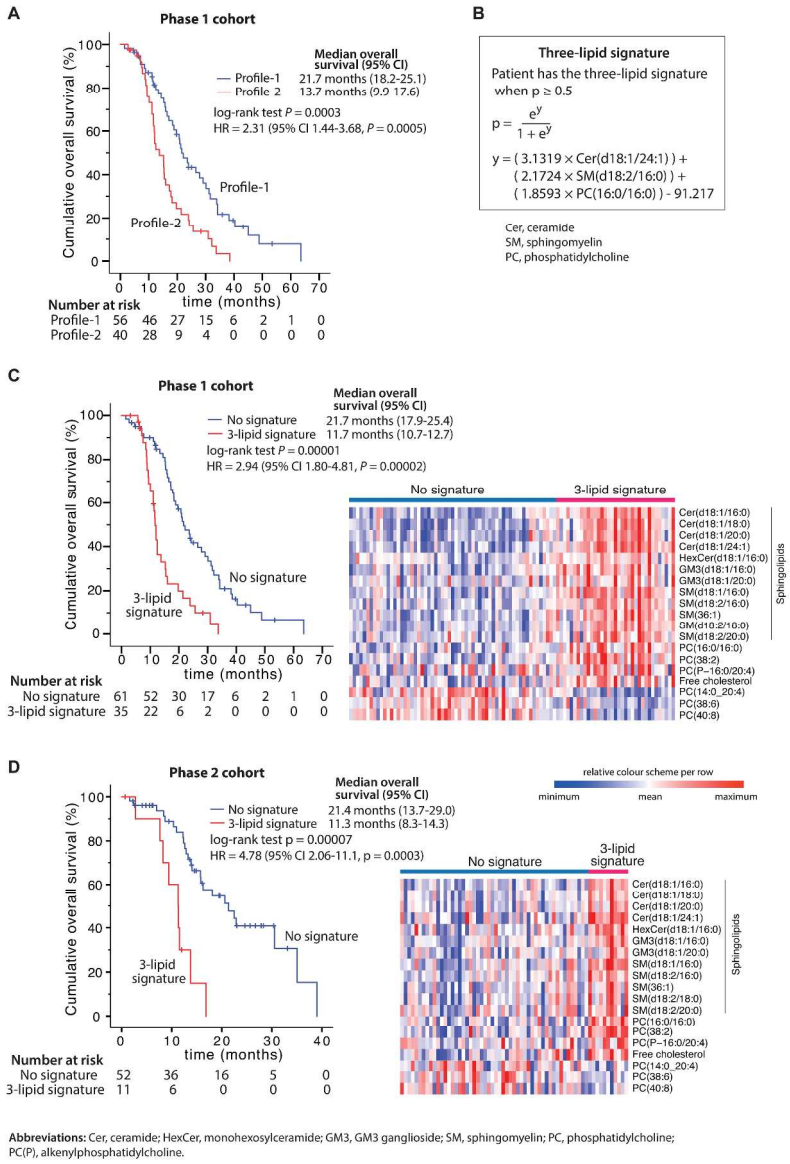


Figure 1. Study schema and data analysis strategy

217x427mm (300 x 300 DPI)

FIGURE 2



Abbreviations: Cer, ceramide; HexCer, monohexosylceramide; GM3, GM3 ganglioside; SM, sphingomyelin; PC, phosphatidylcholine; PC(P), alkenylphosphatidylcholine.

Figure 2. Survival curves, lipid signature, and heatmaps of prognostic baseline plasma lipid levels. (A) Survival curves of Phase 1 discovery cohort classified by latent class analysis of baseline lipidomic profile; (B) Three-lipid signature of normalised baseline lipid levels; (C) Survival curves of Phase 1 discovery cohort classified by the three-lipid signature, and heatmap of 19 prognostic lipids validated in Phase 2; (D) Survival curves of Phase 2 validation cohort classified by the three-lipid signature, and heatmap of the 19 validated prognostic lipids.

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