

Supplementary Figure Legends

Figure S1. Chemorefractory disease is defined as that of patients who progress within 90 days of completion of chemotherapy. Chemosensitive disease is defined as that of patients who progress more than 90 days after the completion of chemotherapy. Chemorefractory disease may be thought of as intrinsic drug resistance whilst the relapse of initially chemosensitive disease is associated with acquired drug resistance. However, chemorefractory and chemosensitive disease refer to the clinical criteria above and intrinsic and acquired drug resistance refer to molecular mechanisms of drug resistance that are incompletely understood in SCLC.

Figure S2. Schematic overview of the bioinformatics development and validation of a copy number profile-based signature predictive of chemo-response. Paired-end sequence reads from the training set (88 CTCs) and CTC Testing set (112 CTCs) were aligned to the human reference genome using BWA. For the CDX Testing set (nine CDX samples), a two-step strategies was adopted to remove potentially contaminating mouse reads. First, sequencing reads were aligned independently to the human and mouse genomes, and then reads that cross-aligned to both genomes were discarded before downstream analysis. Copy number changes in both training and validation sets were determined using CONTROL-FREEC. Predictive features that discriminated between chemosensitive and chemorefractory samples in the training cohort were identified using LIMMA (FDR <1%). Then a Support Vector Machine (SVM)-based classifier was constructed. Model accuracy and stability were estimated using 10-fold cross-validation over 100 iterations. The performance of the classifier was evaluated on samples from the testing CTC set and CDX models.

Figure S3. Example of chromosomal copy number profiles generated from five CTCs and a single WBC from a baseline SCLC blood sample. Following isolation and WGA of each sample low-pass WGS data was generated and analysed using CONTROL-FREEC with gains indicated in red and losses indicated in blue (CTC1-CTC5). WBC controls were included for every patient to confirm tumor specific alterations (sWBC).

Figure S4. Comparison of all genes showing significant frequencies of gain or loss in Rudin *et al.* SCLC tumor data set (2) vs frequencies observed in 88 baseline CTCs and 20 WBC from 13 SCLC patients. Similar levels of gain and loss were seen between the tumor and CTC samples, with no significant changes seen in the WBC controls.

Figure S5. Schematic representation of the chromosomal location of 2281 *loci* within the 16 profiles identified in the CTC CNA classifier. All *loci* within each profile are indicated by a coloured line, with chromosome numbering indicated on the right of the figure. Each profile is represented by a separate colour.

Figure S6. Chromosomal copy number changes of nine CDX models derived from two chemorefractory (purple) and four chemosensitive (green) SCLC patients. Areas of chromosomal gains (red) and loss (blue) based on all protein-coding genes are shown. Location of common SCLC deletions (3p, 17p) and amplification (3q, 5p) are indicated by blue and red arrows. The CTC CNA classifier correctly categorised five of the six patient derived CDX tumours (83.3%). CDX sample names are indicated on the left of the figure and chromosomal location across the top of the figure.

Supplementary Table Legends

Table ST1. Demographics detailing disease status, PS, PFS, OS, time to progression from end of 1st line treatment, chemotherapy received and CTC numbers at baseline for all 31 patients and all 6 CDX patient donors. For five patients used in longitudinal study, CTC numbers at relapse are shown. Patients that received thoracic radiotherapy post-chemotherapy response assessment in the absence of progression are indicated with an asterisk. PS = performance status.

Table ST2. Details of the 16 profiles used in the CTC CNA classifier of chemosensitive and chemorefractory SCLC after grouping of 2281 informative *loci*. Chromosomally amplified

profiles are shown in red, deleted profiles are shown in blue. The chromosomal location and number of genes associated with each profile are shown. Details of the genes associated with each profile are further detailed in Supplementary Table ST6.

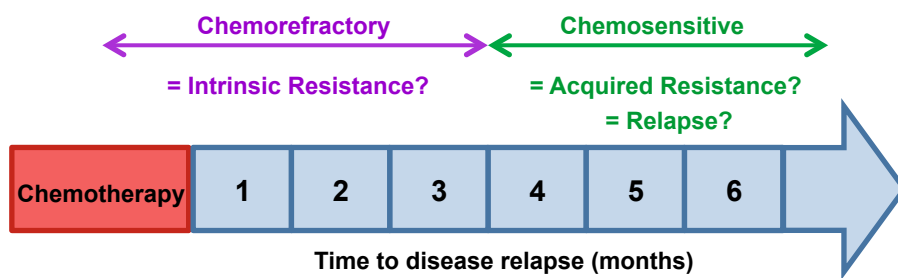
Table ST3. Analysis of intra-patient heterogeneity based on the 16 profile CTC CNA classifier for 31 patients. Green = patients correctly called based on CTC CNA classifier, pink = patients incorrectly called based on CTC CNA classifier.

Table ST4. CTC CNA classifier associations with clinical characteristics for 31 patients as assessed by Fisher's Exact Test showing no significant associations.

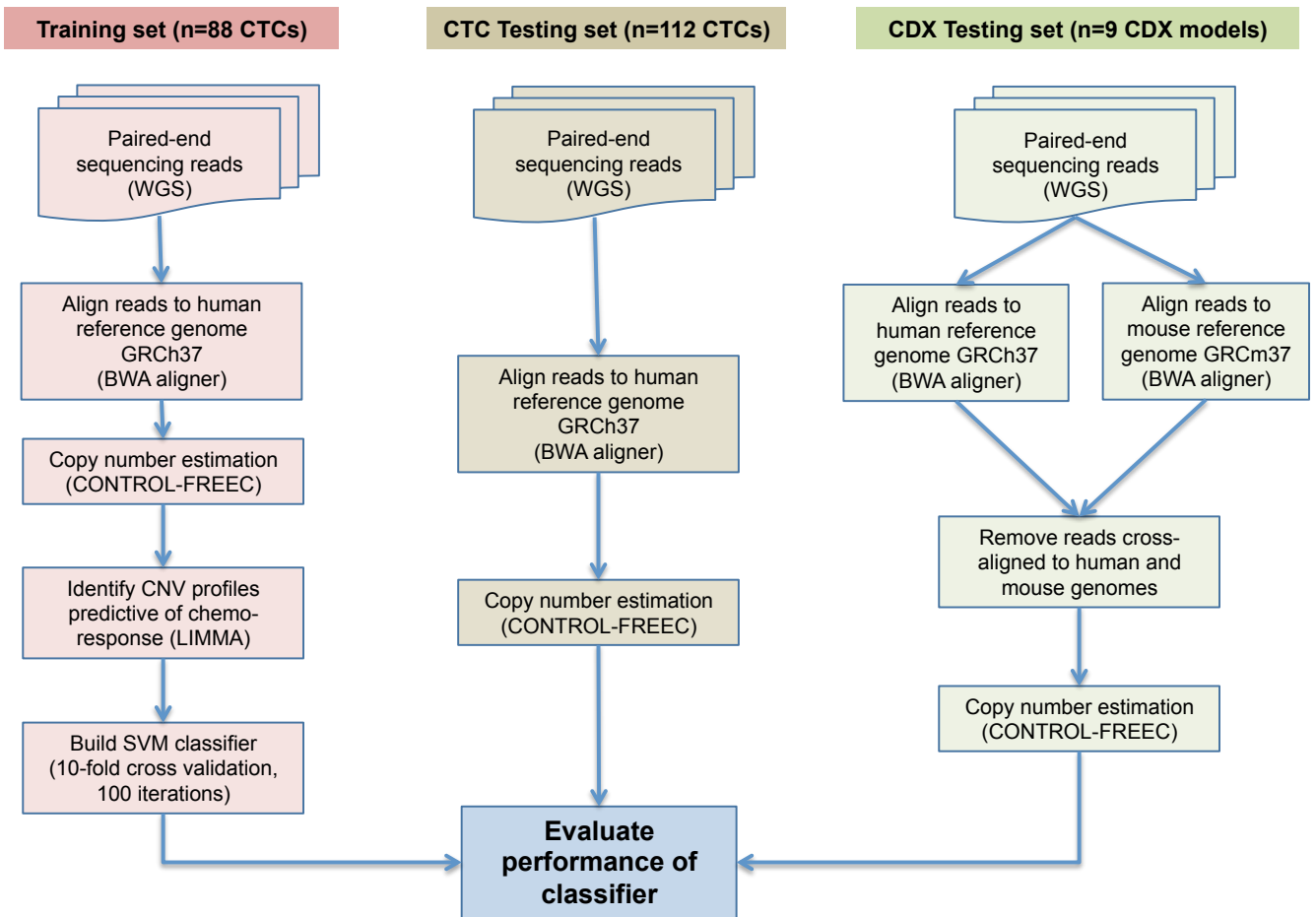
Table ST5. Assignment of CTCs samples at baseline and, for initially chemosensitive patients at relapse, based on the CTC CNA classifier. Clinical classification (clinical response) and CTC CNA classification (predicted response) are shown.

Table ST6. Excel spread sheet of 2281 *loci* identified in 16 profiles of CTC CNA predictive signature.

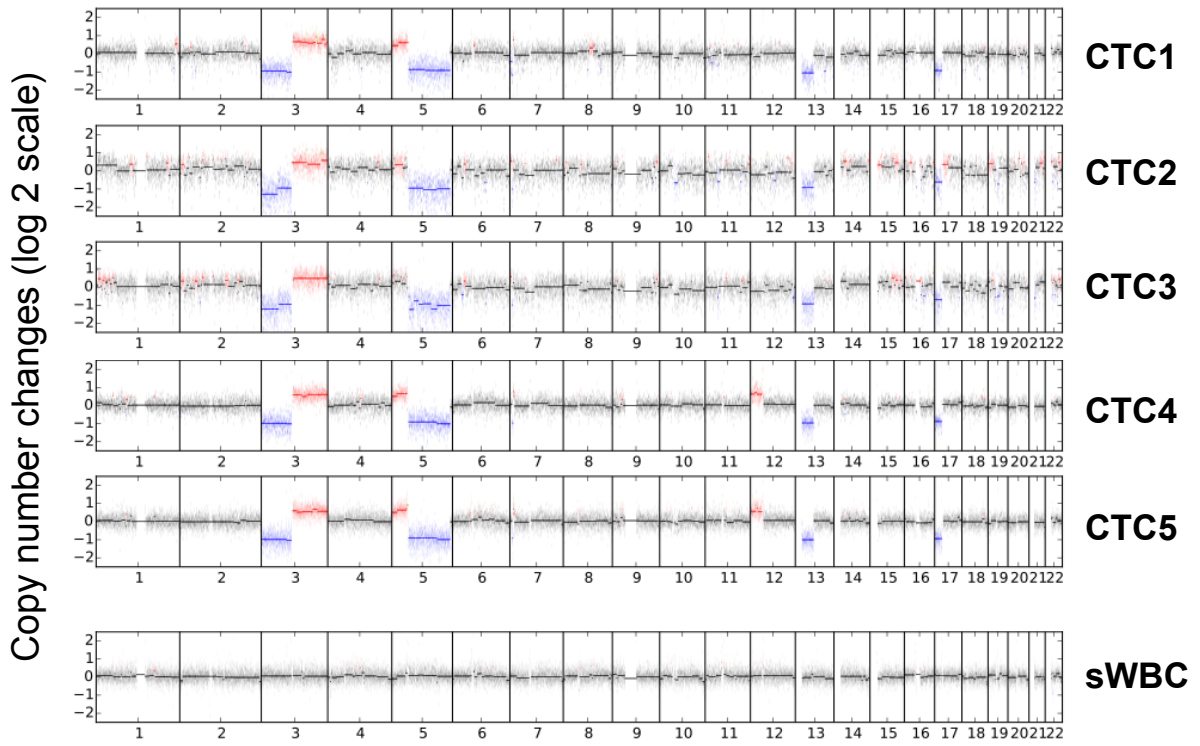
Supplementary Figure S1.



Supplementary Figure S2.

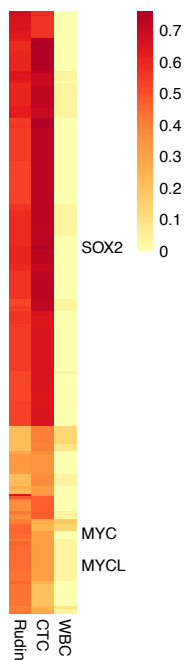


Supplementary Figure S3.

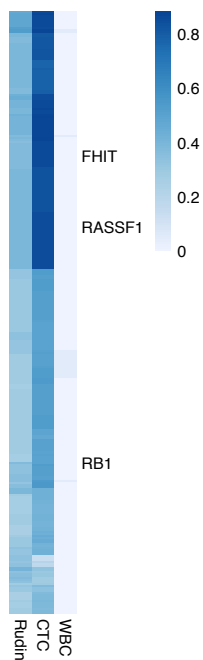


Supplementary Figure S4.

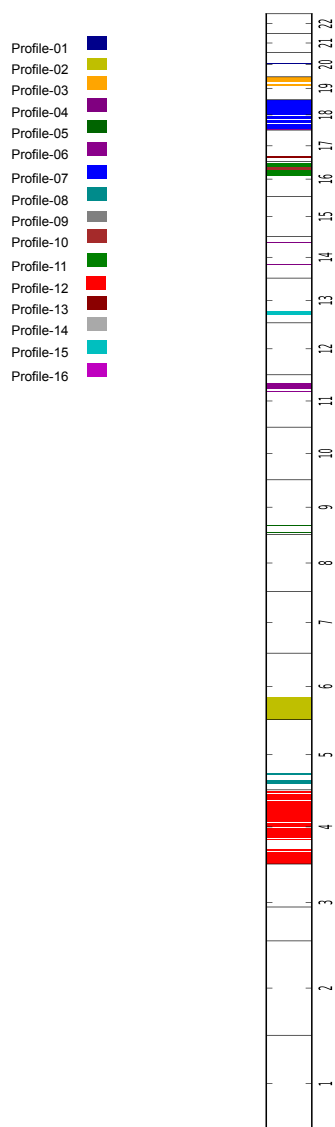
Significant gains seen in SCLC



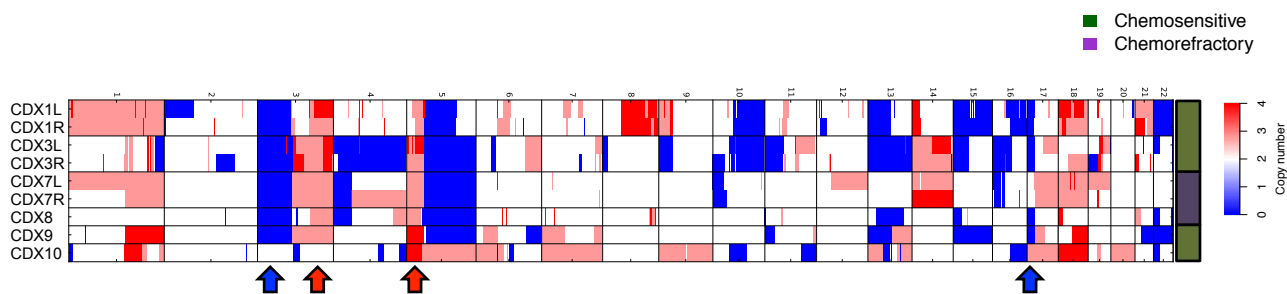
Significant deletions seen in SCLC



Supplementary Figure S5.



Supplementary Figure S6.



Patient	Sample	Patient clinical status	Sample CNA Classification	Patient CNA classification	Accuracy
CDX1	CDX1_L	Sensitive	Sensitive	Sensitive	83.3% (5/6)
	CDX1_R		Sensitive		
CDX3	CDX3_L	Sensitive	Sensitive	Sensitive	
	CDX3_R		Sensitive		
CDX7	CDX7_L	Refractory	Refractory	Refractory	
	CDX7_R		Refractory		
CDX8	CDX8	Refractory	Sensitive	Sensitive	
CDX9	CDX9	Sensitive	Sensitive	Sensitive	
CDX10	CDX10	Sensitive	Sensitive	Sensitive	

Supplementary Table ST1.

Patient number	Chemosensitive/Chemorefractory	Age	Sex	Smoking History (pack years)	PS	Metastatic sites	1st line Chemotherapy	Lines of Chemotherapy	PFS (months)	OS (months)	Time to progression from end of 1st line treatment	CTC count at baseline	CTC count at Relapse
P1	Chemosensitive	73	Male	30	3	lymph node, bone, adrenal	carboplatin	1	5.1	6.6	3.5	522	1126
P2	Chemosensitive	57	Female	64	2	lymph node, bone, liver	carboplatin and etoposide	2	8.1	14.3	6.0	1200	578
P3	Chemosensitive	59	Male	40	1	liver, pleural effusion	carboplatin and etoposide	2	6.2	10.5	3.8	250	12
P4	Chemosensitive	68	Female	67.5	2	lymph node, liver	carboplatin and etoposide	3	6.2	16.2	3.4	27	289
P5	Chemosensitive	63	Female	23	2	bone, liver	carboplatin and etoposide	2	6.1	7.6	3.8	307	312
P6	Chemosensitive	71	Female	40	1	bone, liver	carboplatin and etoposide	1	9.5	9.6	7.4	836	NA
P7	Chemosensitive	65	Male	60	3	adrenal, lung	carboplatin	1	5.6	6.9	3.3	3780	NA
P8	Chemorefractory	61	Male	90	1	lymph node, bone, liver, soft tissue	carboplatin and etoposide	1	2.8	4.4	0.3	20815	NA
P9	Chemorefractory	65	Female	84	2	bone, liver, brain, pleural effusion	carboplatin and etoposide	1	3.1	3.5	2.2	1625	NA
P10	Chemorefractory	78	Female	27	2	lymph node, liver, pleura	carboplatin	1	0.7	0.9	0.7	1376	NA
P11	Chemorefractory	69	Female	20	3	lymph node, bone, liver, pleura	carboplatin	1	2.8	5.3	0.5	204	NA
P12	Chemorefractory	78	Male	43	3	liver, bone	carboplatin	1	2.1	3.1	0.5	370	NA
P13	Chemorefractory	80	Male	30	2	lymph node, liver	carboplatin	1	1.0	1.5	1.0	1018	NA
P14	Chemosensitive	64	Female	100	2	pleura	carboplatin and etoposide	1	1.1	1.1	Non cancer related cause of death	1356	NA
P15	Chemosensitive	70	Male	130	2	lymph node, pleural effusion	carboplatin and etoposide	1	3.7	4.0	Non cancer related cause of death	170	NA
P16	Chemosensitive	60	Male	Ex Smoker - pack years not recorded	2	lymph node, adrenal	carboplatin and etoposide	1	6.0	11.3	4.6	237	NA
P17	Chemosensitive	82	Female	Ex Smoker - pack years not recorded	1	lymph node, adrenal, spleen	carboplatin and etoposide	1	8.9	11.4	6.7	4061	NA
P18	Chemosensitive	71	Male	40	1	lymph node, bone, liver, adrenal	carboplatin and etoposide	2	8.9	16.1	6.6	377	NA
P19	Chemosensitive	67	Female	25	2	lymph node, liver, adrenal, pancreas	carboplatin	1	1.7	1.7	Non cancer related cause of death	222	NA
P20	Chemosensitive	75	Male	58	2	bone marrow	carboplatin	1	2.6	2.6	Non cancer related cause of death	23243	NA
P21	Chemosensitive	35	Male	40	1	lymph node, bone, liver, pleural effusion	carboplatin and etoposide*	1	5.2	6.7	3.1	109	NA
P22	Chemosensitive	68	Female	26	1	lymph node, bone	carboplatin and etoposide	1	5.1	8.7	3.2	2048	NA
P23	Chemosensitive	58	Female	80	1	brain, adrenal	carboplatin and etoposide	1	6.1	6.1	3.1	44	NA
P24	Chemosensitive	72	Female	40	1	liver, lung	carboplatin and etoposide	1	6.5	12.9	4.4	35	NA
P25	Chemosensitive	50	Female	25	2	lymph node, bone, liver	carboplatin and etoposide*	2	9.1	15.7	7.0	669	NA
P26	Chemosensitive	68	Female	30	1	lymph node, liver, lung	carboplatin and etoposide*	1	4.9	4.9	Not progressed (>3.0 months)	58	NA
P27	Chemosensitive	75	Male	30	3	lymph node, pleura	carboplatin	1	1.0	1.0	Non cancer related cause of death	207	NA
P28	Chemorefractory	74	Male	59	1	liver, pleural effusion	carboplatin and etoposide	1	3.8	4.5	1.1	7687	NA
P29	Chemorefractory	78	Male	20	2	bone, liver	carboplatin	1	2.2	4.5	0.1	43	NA
P30	Chemorefractory	66	Female	40	3	lymph node, liver, adrenal	carboplatin and etoposide	1	4.2	4.5	1.8	1187	NA
P31	Chemorefractory	72	Female	42	1	liver	carboplatin	1	1.6	3.6	0.0	11	NA
CDX1	Chemosensitive	55	Male	40	2	lymph node, bone	carboplatin and etoposide*	1	7.3	7.3	7.3	458	NA
CDX3	Chemosensitive	70	Male	58	3	lymph node	carboplatin and etoposide*	1	8	9.8	7.1	507	NA
CDX7	Chemorefractory	69	Female	40	2	bone, liver, lung	carboplatin	1	4.9	5.2	2.3	901	NA
CDX8	Chemorefractory	63	Female	42	3	bone, liver	carboplatin	1	5.1	6.1	2.3	388	NA
CDX9	Chemosensitive	69	Female	40	2	lymph node, bone, liver, adrenal	carboplatin and etoposide	1	1.7	1.7	Non cancer related cause of death	4660	NA
CDX10	Chemosensitive	75	Male	55	2	lymph node, bone, liver	carboplatin and etoposide	2	10.4	18	8.3	160	NA

* Thoracic radiotherapy given post chemotherapy response assessment in the absence of progression

Supplementary Table ST2.

Segment	Chromosome	Gene Count	Ave copy number Sensitive	Ave copy number Refractory
Profile-1	20	37	2	3
Profile-2	6	258	2	3
Profile-3	19	257	2	3
Profile-4	14	6	2	3
Profile-5	9	11	3	2
Profile-6	11	33	3	2
Profile-7	18	256	3	2
Profile-8	18	18	4	2
Profile-9	5	22	3	2
Profile-10	16	57	3	1
Profile-11	16	155	3	2
Profile-12	16	258	2	1
Profile-13	4	29	1	2
Profile-14	17	3	1	2
Profile-15	3	38	2	1
Profile-16	13	3	2	1

Supplementary Table ST3.

Patient	Clinical classification	CTC CNA classification	Total number CTCs	Number CTCs correctly classified	Number CTCs incorrectly classified	% CTC Heterogeneity	
P1	Chemosensitive	Chemosensitive	7	7	0	0	Homogeneous CTC Classification
P2	Chemosensitive	Chemosensitive	9	9	0	0	
P3	Chemosensitive	Chemosensitive	5	5	0	0	
P4	Chemosensitive	Chemosensitive	4	4	0	0	
P5	Chemosensitive	Chemosensitive	11	11	0	0	
P7	Chemosensitive	Chemosensitive	8	8	0	0	
P14	Chemosensitive	Chemosensitive	6	6	0	0	
P15	Chemosensitive	Chemosensitive	8	8	0	0	
P16	Chemosensitive	Chemosensitive	5	5	0	0	
P17	Chemosensitive	Chemosensitive	7	7	0	0	
P18	Chemosensitive	Chemorefractory	6	0	6	0	
P24	Chemosensitive	Chemosensitive	4	4	0	0	
P8	Chemorefractory	Chemorefractory	9	9	0	0	
P9	Chemorefractory	Chemorefractory	6	6	0	0	
P10	Chemorefractory	Chemorefractory	3	3	0	0	
P11	Chemorefractory	Chemorefractory	7	7	0	0	
P12	Chemorefractory	Chemorefractory	7	7	0	0	
P13	Chemorefractory	Chemorefractory	7	7	0	0	
P28	Chemorefractory	Chemosensitive	5	0	5	0	
P6	Chemosensitive	Chemosensitive	5	4	1	20	Heterogeneous CTC Classification
P19	Chemosensitive	Chemosensitive	5	3	2	40	
P20	Chemosensitive	Chemosensitive	5	3	2	40	
P21	Chemosensitive	Chemosensitive	9	6	3	33	
P22	Chemosensitive	Chemosensitive	9	8	1	11	
P23	Chemosensitive	Chemosensitive	6	4	2	33	
P25	Chemosensitive	Chemosensitive	13	9	4	31	
P26	Chemosensitive	Chemorefractory	4	1	3	25	
P27	Chemosensitive	Chemosensitive	7	6	1	14	
P29	Chemorefractory	Chemorefractory	3	2	1	33	
P30	Chemorefractory	Chemorefractory	7	4	3	43	
P31	Chemorefractory	Chemorefractory	3	2	1	33	

Supplementary Table ST4.

	CTC CNA Classifier	
	Chemosensitive	Chemorefractory
Age at baseline, years, median (range)	67.5 (35 - 82)	71 (61 - 80)
Mann-Whitney's P	p=0.098	
Sex		
Female	11	6
Male	9	5
Fisher's exact P	p=1	
Performance Status		
Group 1: 1	8	4
Group 2: 2-3	12	7
Fisher's exact P	p=1	
Treatment		
Carboplatin	5	6
Carboplatin and etoposide	15	5
Fisher's exact P	p=0.1318	
Number Metastatic Sites, rounded mean (range)	2 (1-4)	3 (1-4)
Mann-Whitney's P	p=0.088	
CTC count at baseline, rounded median (range)	414 (27-23243)	377 (11-20815)
Mann-Whitney's P	P=0.855	

Supplementary Table ST5.

Chemosensitive Baseline							Chemorefractory Baseline				
Pre-Treatment			Relapse			Pre-Treatment					
Patient	Cellular Sample	Clinical response	Predicted response	Cellular Sample	Clinical Response	Predicted response	Patient	Cellular Sample	Clinical response	Predicted response	
P1	P1 CTC-01	Chemosensitive	Chemosensitive	P1 CTC-09	Relapse	Chemosensitive	P8	P8 CTC-01	Chemorefractory	Chemorefractory	
	P1 CTC-02	Chemosensitive	Chemosensitive	P1 CTC-10	Relapse	Chemosensitive		P8 CTC-02	Chemorefractory	Chemorefractory	
	P1 CTC-03	Chemosensitive	Chemosensitive	P1 CTC-11	Relapse	Chemosensitive		P8 CTC-03	Chemorefractory	Chemorefractory	
	P1 CTC-04	Chemosensitive	Chemosensitive	P1 CTC-12	Relapse	Chemosensitive		P8 CTC-04	Chemorefractory	Chemorefractory	
	P1 CTC-05	Chemosensitive	Chemosensitive	P1 CTC-13	Relapse	Chemosensitive		P8 CTC-05	Chemorefractory	Chemorefractory	
	P1 CTC-06	Chemosensitive	Chemosensitive	P1 CTC-14	Relapse	Chemosensitive		P8 CTC-06	Chemorefractory	Chemorefractory	
	P1 CTC-07	Chemosensitive	Chemosensitive	P1 CTC-15	Relapse	Chemosensitive		P8 CTC-07	Chemorefractory	Chemorefractory	
P2	P2 CTC-01	Chemosensitive	Chemosensitive	P2 CTC-16	Relapse	Chemosensitive	P8 CTC-08	Chemorefractory	Chemorefractory		
	P2 CTC-02	Chemosensitive	Chemosensitive	P2 CTC-17	Relapse	Chemosensitive	P8 CTC-09	Chemorefractory	Chemorefractory		
	P2 CTC-03	Chemosensitive	Chemosensitive	P2 CTC-18	Relapse	Chemosensitive	P9 CTC-01	Chemorefractory	Chemorefractory		
	P2 CTC-04	Chemosensitive	Chemosensitive	P2 CTC-07	Relapse	Chemosensitive	P9 CTC-02	Chemorefractory	Chemorefractory		
	P2 CTC-05	Chemosensitive	Chemosensitive	P2 CTC-08	Relapse	Chemosensitive	P9 CTC-03	Chemorefractory	Chemorefractory		
	P2 CTC-06	Chemosensitive	Chemosensitive	P2 CTC-09	Relapse	Chemosensitive	P9 CTC-04	Chemorefractory	Chemorefractory		
	P2 CTC-07	Chemosensitive	Chemosensitive	P2 CTC-10	Relapse	Chemosensitive	P9 CTC-05	Chemorefractory	Chemorefractory		
P3	P3 CTC-01	Chemosensitive	Chemosensitive	P3 CTC-11	Relapse	Chemosensitive	P10	P10 CTC-01	Chemorefractory	Chemorefractory	
	P3 CTC-02	Chemosensitive	Chemosensitive	P3 CTC-12	Relapse	Chemosensitive	P10 CTC-02	Chemorefractory	Chemorefractory		
	P3 CTC-03	Chemosensitive	Chemosensitive	P3 CTC-13	Relapse	Chemosensitive	P10 CTC-03	Chemorefractory	Chemorefractory		
	P3 CTC-04	Chemosensitive	Chemosensitive	P3 CTC-07	Relapse	Chemorefractory	P11 CTC-01	Chemorefractory	Chemorefractory		
	P3 CTC-05	Chemosensitive	Chemosensitive	P3 CTC-08	Relapse	Chemorefractory	P11 CTC-02	Chemorefractory	Chemorefractory		
P4	P4 CTC-01	Chemosensitive	Chemosensitive	P4 CTC-09	Relapse	Chemorefractory	P11	P11 CTC-03	Chemorefractory	Chemorefractory	
	P4 CTC-02	Chemosensitive	Chemosensitive	P4 CTC-10	Relapse	Chemorefractory		P11 CTC-04	Chemorefractory	Chemorefractory	
	P4 CTC-03	Chemosensitive	Chemosensitive	P4 CTC-11	Relapse	Chemorefractory		P11 CTC-05	Chemorefractory	Chemorefractory	
	P4 CTC-04	Chemosensitive	Chemosensitive	P4 CTC-12	Relapse	Chemorefractory		P11 CTC-06	Chemorefractory	Chemorefractory	
	P5	P5 CTC-01	Chemosensitive	Chemosensitive	P5 CTC-13	Relapse		Chemorefractory	P12 CTC-07	Chemorefractory	Chemorefractory
		P5 CTC-02	Chemosensitive	Chemosensitive	P5 CTC-14	Relapse		Chemorefractory	P12 CTC-05	Chemorefractory	Chemorefractory
		P5 CTC-03	Chemosensitive	Chemosensitive	P5 CTC-15	Relapse		Chemorefractory	P12 CTC-06	Chemorefractory	Chemorefractory
P5 CTC-04		Chemosensitive	Chemosensitive	P5 CTC-16	Relapse	Chemorefractory	P12 CTC-07	Chemorefractory	Chemorefractory		
P5 CTC-05		Chemosensitive	Chemosensitive	P5 CTC-17	Relapse	Chemorefractory	P12 CTC-08	Chemorefractory	Chemorefractory		
P5 CTC-06		Chemosensitive	Chemosensitive	P5 CTC-18	Relapse	Chemorefractory	P12 CTC-09	Chemorefractory	Chemorefractory		
P5 CTC-07		Chemosensitive	Chemosensitive	P5 CTC-19	Relapse	Chemorefractory	P12 CTC-10	Chemorefractory	Chemorefractory		
P5 CTC-08	Chemosensitive	Chemosensitive	P5 CTC-20	Relapse	Chemorefractory	P13 CTC-01	Chemorefractory	Chemorefractory			
P5 CTC-09	Chemosensitive	Chemosensitive	P5 CTC-21	Relapse	Chemorefractory	P13 CTC-02	Chemorefractory	Chemorefractory			
P5 CTC-10	Chemosensitive	Chemosensitive	P5 CTC-22	Relapse	Chemorefractory	P13 CTC-03	Chemorefractory	Chemorefractory			
P5 CTC-11	Chemosensitive	Chemosensitive	P5 CTC-23	Relapse	Chemorefractory						