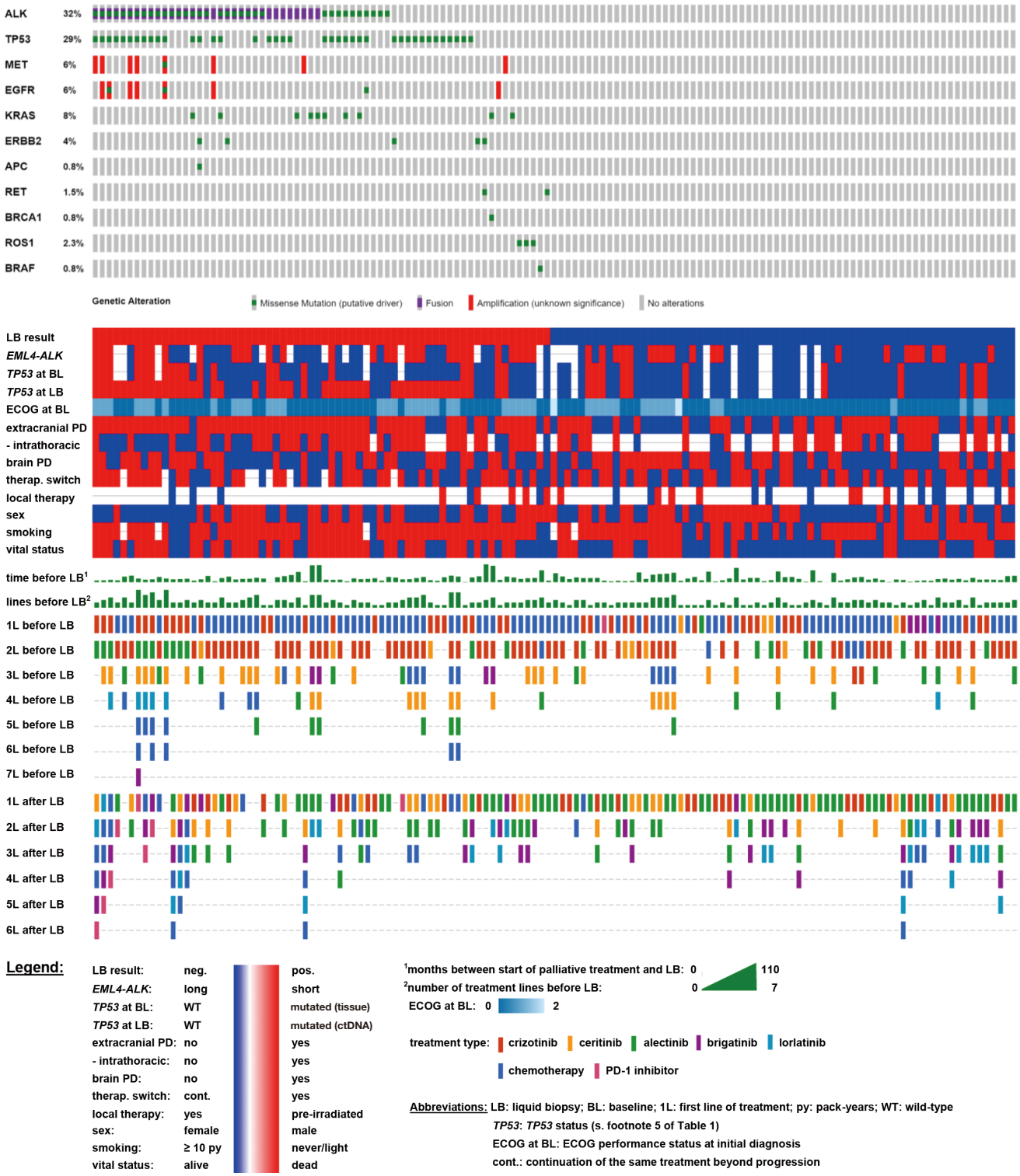

Figure S1 Oncoprint plot of ctDNA findings and patient characteristics.

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patient #	TP53 result in tissue (baseline)			TP53 result in ctDNA (liquid rebiopsies)		
	mutation	AF (%)	affected exon	mutation	AF (%)	affected exon
1	c.991C>T, p.Q331* c.404G>T, p.C135F	10 12	9 5	---		
2	c.722C>G, p.S241C	48	7	c.722C>G, p.S241C c.730G>T, p.G244C c.714T>A, p.C238*	0.17%-0.41% 0.10% 0.10%	7 7 7
3	---			c.711G>A, p.M237I	0.15%-0.29%	7
4	c.581T>C, p.L194P	14	6	c.581T>C, p.L194P c.1009C>T, p.R337C	0.09% 0.34%-3.60%	6 11
5	---			---		
6	---			c.415A>G, p.K139E c.451C>T, p.P151S	0.15%-0.30% 0.79%	5 5
7	---			c.827C>A, p.A276D	0.64%	8
8	---			---		
9	---			---		
10	n/a			---		
11	---			c.584T>C, p.I195T c.646G>T, p.V216L c.653T>G, p.V218G c.707A>G, p.Y236C c.712T>A, p.C238S	0.12%-0.29% 0.11%-0.23% 0.03% 0.09% 0.07%	6 6 6 7 7
12	---			c.641A>G, p.H214R	0.07%	6
13	n/a			---		
14	---			---		
15	---			---		
16	---			---		
17	c.817C>T, p.R273C	23	8	---		
18	c.745A>T, p.R249W	45	7	c.745A>T, p.R249W	1.05%-2.99%	7
19	---			c.374C>G, p.T125R c.715A>G, p.N239D c.994-2A>G, p.spl?	0.91%-3.03% 0.12% 0.24%-0.52%	4 7 11
20	c.454C>T, p.P152S	20	5	---		
21	---			---		
22	---			---		
23	---			---		
24	---			---		
25	---			c.742C>T, p.R248W	0.12%-0.78%	7
26	---			---		
27	c.920-2A>G, p.spl?	13	9	c.920-2A>G, p.spl?	0.42%-12.38%	9
28	---			---		
29	c.195-196insCAGA, p.M66fs*84	33	4	c.676G>T, p.G226C	0.08%	7
30	---			---		
31	---			---		
32	---			---		
33	---			---		
34	---			---		
35	---			---		
36	c.578A>G, p.H193R	17	6	---		
37	---			---		
38	---			---		
39	---			c.472C>A, p.R158S	0.09%	5
40	---			---		
41	---			---		
42	---			c.708C>A, p.Y236*	0.14%	7
43	c.659A>G, p.Y220C	16	6	c.659A>G, p.Y220C	0.13-5.48%	6
44	---			---		
45	---			---		
46	n/a			---		
47	n/a			---		
48	c.375+1G>A, p.spl?	14	4	---		
49	c.548C>G, p.S183*	24	5	---		
50	n/a			---		
51	n/a			---		
52	n/a			---		
53	c.574C>T, p.Q192*	13	6	---		
54	---			---		
55	n/a			---		
56	n/a			---		

AF: allelic frequency; ---: no mutation detected; n/a: not available

Figure S2 *TP53* mutations detected in the study patients. *TP53* mutations identified at baseline using tissue NGS, and at progression in ctDNA of study patients. Concordant results are highlighted in green (n=5), *TP53* mutations that were newly detectable at progression in orange (n=9), and *TP53* mutations detectable only in tissue at baseline in blue (n=7). In a single case, different *TP53* mutations were detected at baseline and at progression (n=1, in grey). AF, allelic frequency; –, no mutation detected; n/a, not available.

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Table S1 Treatment and response of patients with ALK mutations

#	Previous treatment	ALK mutation(s)	Subsequent treatment	TKI sensitivity	Best response
1	Crizotinib, alectinib	G1202R+L1196M	Ceritinib, lorlatinib, CHT, brigatinib	No	SD
2	Crizotinib, alectinib, ceritinib	G1202R+L1196M+V1149A	Lorlatinib, CHT, brigatinib	No	SD
3	Crizotinib, alectinib, ceritinib, lorlatinib, brigatinib	G1202R+L1196M	CHT, brigatinib	No	PD
4	Crizotinib, alectinib, ceritinib, lorlatinib, brigatinib, CHT	G1202R+L1196M	–	No	–
5	Crizotinib, alectinib	I1171T+V1180L	Alectinib, ceritinib, brigatinib, CHT, lorlatinib	No	PD
6	Crizotinib, alectinib	I1171T+L1196M	Ceritinib, brigatinib, CHT, lorlatinib	No	PD
7	CHT, crizotinib	F1174C+G1269A+F1174L	Crizotinib, ceritinib, alectinib	No	PD
8	CHT, crizotinib	L1196M+E1129V	Crizotinib, ceritinib, alectinib	No	PD
9	Crizotinib	L1196M+E1129V	Ceritinib, alectinib	No	SD
10	CHT, crizotinib, ceritinib	G1202R+R1192P+F1174C	CHT	No	PD
11	CHT, crizotinib, ceritinib, CHT	G1202R+R1192P+F1174C +G1128A+S1206A +T1151K+G1128A	–	No	–
12	CHT, crizotinib	G1269A+L1187P	Crizotinib, alectinib, CHT	No	PD
13	CHT, crizotinib	F1174C+G1269A+F1174L	–	No	–
14	CHT, crizotinib	I1057T+Q453E	Ceritinib, CHT, alectinib	No	PD
15	Crizotinib, ceritinib, alectinib, lorlatinib	L1196M	CHT, brigatinib	Yes	SD
16	CHT, crizotinib	L1196M	Ceritinib, alectinib	Yes	SD
17	Crizotinib, alectinib, ceritinib, lorlatinib, CHT	L1196M	CHT, brigatinib	Yes	SD
18	Crizotinib, alectinib, ceritinib, lorlatinib, CHT	L1196M	Brigatinib	Yes	SD
19	Crizotinib, alectinib, ceritinib	L1196M	Brigatinib, CHT, lorlatinib	Yes	SD
20	Crizotinib, ceritinib, CHT	D1203N	Alectinib	Yes	PD
21	CHT, crizotinib	F1174V	Alectinib	Yes	PD
22	CHT, crizotinib	G839R	Alectinib	Yes	PR
23	Crizotinib, ceritinib, alectinib	G1128R	Brigatinib	Yes	PR
24	Crizotinib, ceritinib, alectinib	G1128A	Brigatinib	Yes	PR
25	CHT	R557H	Crizotinib	Yes	PR
26	CHT	S1081R	Alectinib	Yes	PR
27	Crizotinib	C1235R	Crizotinib, alectinib	Yes	SD
28	Crizotinib	S619F	Crizotinib, alectinib	Yes	SD
29	CHT, crizotinib	W1320C	Ceritinib	Yes	SD
30	CHT, crizotinib, alectinib, CHT	G1202R	–[1]	Yes	–
31	CHT, crizotinib, alectinib	G1202R	CHT [1]	Yes	PD
32	CHT, crizotinib	I1057T	Crizotinib, ceritinib, CHT, alectinib	Unknown	PD
33	CHT, crizotinib, ceritinib	I1057T	CHT, alectinib	Unknown	PD
34	CHT, crizotinib, ceritinib, CHT, alectinib	T1041S	–	Unknown	–

The type of *ALK* mutation together with previous and subsequent treatments for all 34 cases with ALK mutated ctDNA shown in suppl. Figure 1 are detailed here, because these can potentially be treated within the routine setting. However, 14/34 (41%) cases (#1–14 in the Table below) had coexistence of multiple *ALK* mutations, which is associated with resistance to available ALK inhibitors (1). 15/34 (#15–31) were sensitive to the ALK TKI actually administered subsequently to the patient, according to the literature and/or the clinical benefit observed (2). Two cases (2/34, #30–31) had the G1202R mutation, but did not receive lorlatinib, because it was not available at the time of patient treatment, while in 3 other cases (#32–34) TKI sensitivity of the detected *ALK* mutations is unclear. Overall, 52% (34/66) of instances with positive LB showed *ALK* mutations, and 17/34 (50%) of them were treatable, which corresponds to treatable alterations in 26% (17/66) of LB-positive cases in our study. [1], lorlatinib was not available at the time of patient treatment (was approved by the EMA in May 2019). CHT, chemotherapy; PD, progressive disease; PR, partial response; SD, stable disease.

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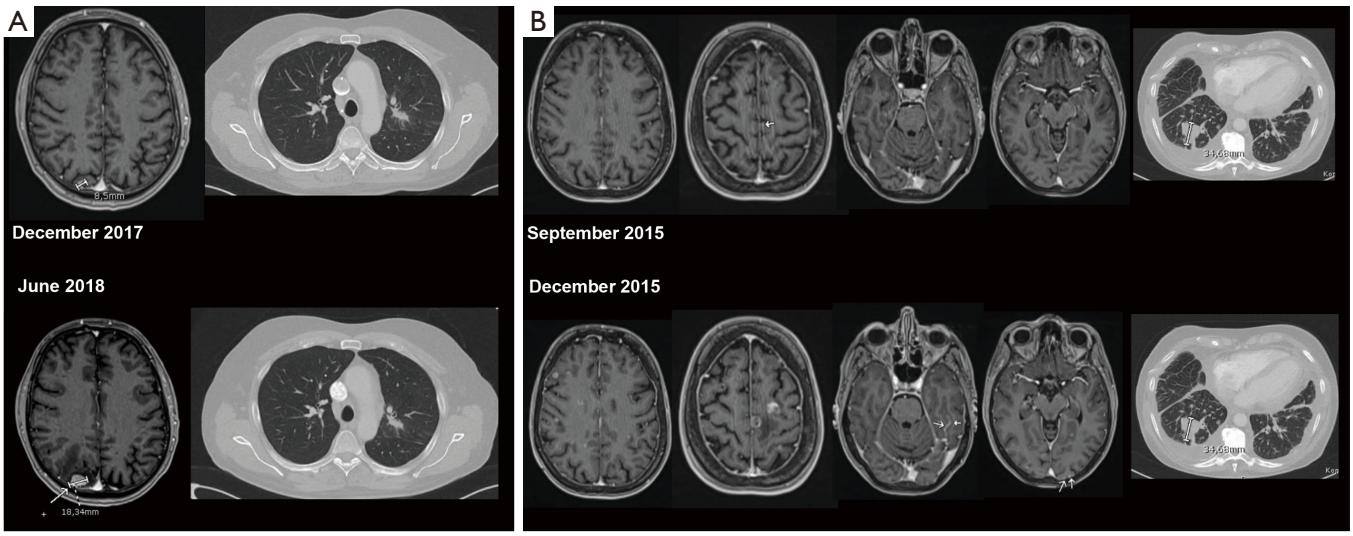

Figure S3 Exemplary cases with CNS-only progression and positive blood liquid biopsies. Two exemplary cases with brain-only progression and positive blood liquid biopsies. In patient A, growth of an occipital metastasis from <1 to 1.8 cm with meningeal contact (images on the left) was associated with detectable ctDNA in the blood (*BRAF* V600E with VAF 0.09%, and *KEAP1* R459Q with VAF 0.32%), while previous liquid biopsies of the same patient were negative. Extracranial tumor manifestations were stable (chest CT images on the right). In patient B, appearance of diffuse intracerebral lesions and multifocal meningeal carcinomatosis in December 2015 (partly depicted in the lower images, meningeal carcinomatosis shown with arrows) was associated with emergence of *ALK* F1174L (VAF 0.88%), and *ALK* G1269A (VAF 0.18%) mutations not detectable in the previous sample of September 2015 (upper images). Extracranial disease was stable.

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