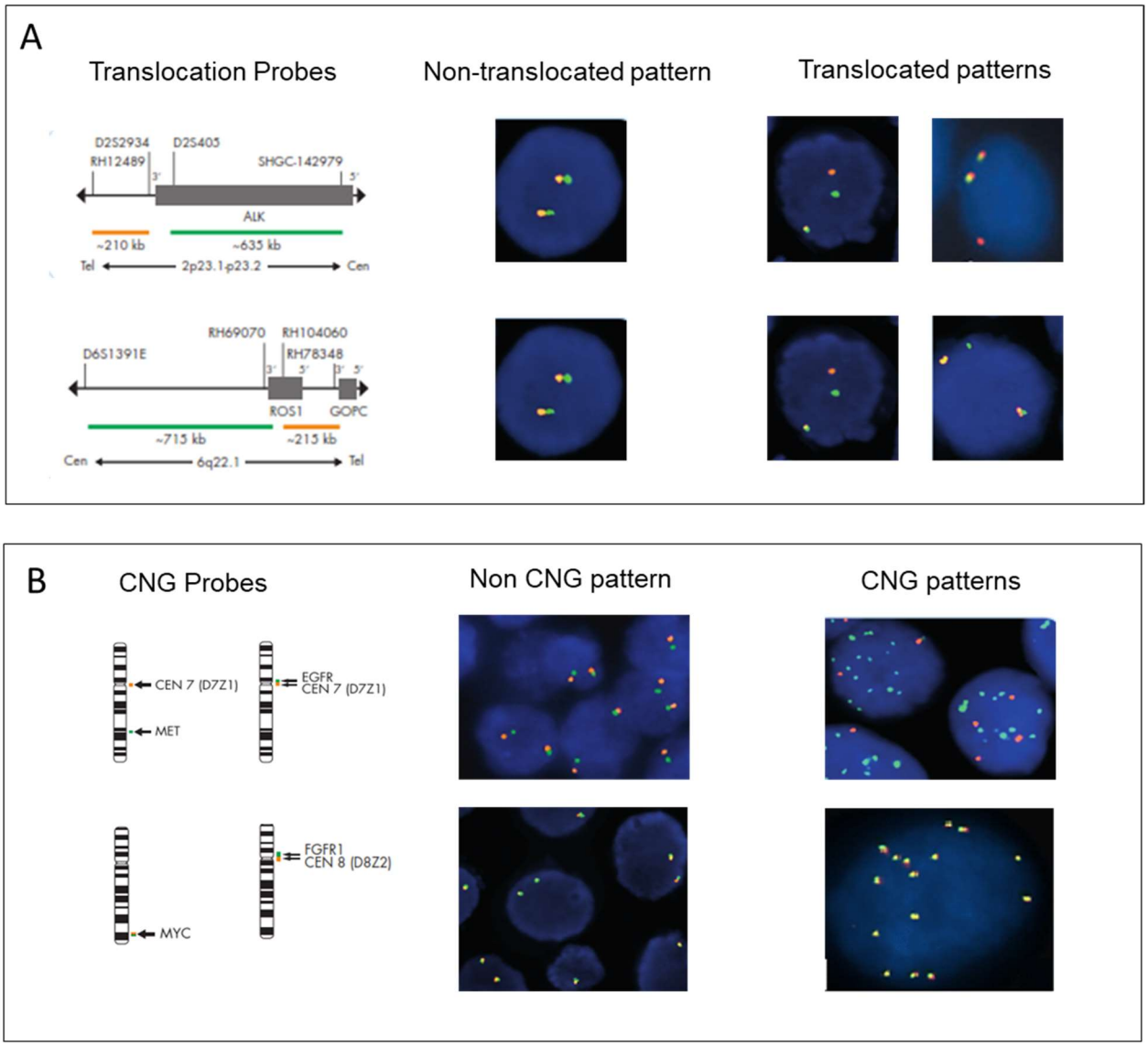

Figure S1: (A) Design of ALK and ROS1 probes used for translocation studies and diagrams of the patterns observed in translocated and non-translocated interphase cells. (B) Design of *MET*/CEN7, *EGFR*/CEN7, *FGFR1*/CEN8 and *MYC* probes used for CNG assessment and diagrams of interphase cells with and without CNG patterns.

Table S1: FISH probe designs and signal patterns. NA: True amplification can not be assessed with ZytoLight ® SPEC MYC Dual Color Break Apart.

TRANSLOCATION PROBES	DESIGN	Non-translocated (negative) pattern	Translocated (positive) patterns
ZytoLight ® SPEC ALK Dual Color Break Apart	3' end Orange / 5' end Green	Fusion signals (Yellow)	Split pattern: separate green and orange signals Split pattern with loss of 5' end : separate orange signals
ZytoLight® SPEC ROS1 Dual Color Break	3' end Green / 5' end Orange	Fusion signals (Yellow)	Split pattern: separate green and orange signals Split pattern with loss of 5' end : separate green signals

COPY NUMBER GAIN PROBES	DESIGN	Non-CNG pattern	CNG pattern	True Amplification
ZytoLight ® SPEC MET/CEN 7 Dual Color	locus green /centromer Orange	<5 green signals	≥ 5 green signals	ratio green/red signals >2
ZytoLight ® SPEC FGFR1/CEN 8 Dual Color	locus green /centromer Orange	<5 green signals	≥ 5 green signals	ratio green/red signals >2
ZytoLight ® SPEC EGFR/CEN 7 Dual Color	locus green /centromer Orange	<5 green signals	≥ 5 green signals	ratio green/red signals >2
ZytoLight ® SPEC MYC Dual Color Break Apart	3' end Green / 5' end Orange	<5 yellow signals	≥ 5 yellow signals	NA

Table S2: Further details on percentage of cells with alterations and number of interphase cells counted for each sample passage.

Sample	Passage	Alteration	% Translocated/amplified cells	Number of interphase cells counted
Sample 1	2	*ALK* fusion	50	73
Sample 1	3	*ALK* fusion	17	55
Sample 1	5	*ALK* fusion	50	53
Sample 1	8	*ALK* fusion	90	100
Sample 1	9	*ALK* fusion	75	58
Sample 1	11	*ALK* fusion	65	84
Sample 1	13	*ALK* fusion	50	69
Sample 2	2	*ALK* fusion	50	61
Sample 2	3	*ALK* fusion	72	58
Sample 2	5	*ALK* fusion	37	52
Sample 11	1	*ROS1* fusion	9	181
Sample 11	2	*ROS1* fusion	87	98
Sample 11	5	*ROS1* fusion	78	83
Sample 11	7	*ROS1* fusion	99	120
Sample 12 AC	1	*ROS1* fusion	76	190
Sample 12 AC	2	*ROS1* fusion	99	110
Sample 12 AC	5	*ROS1* fusion	100	100
Sample 12 FC	1	*ROS1* fusion	100	112
Sample 12 FC	2	*ROS1* fusion	100	119
Sample 12 FC	5	*ROS1* fusion	100	110
Sample 1	5	*MET* CNG	77	30
Sample 1	8	*MET* CNG	50	60
Sample 1	9	*MET* CNG	53	60
Sample 1	11	*MET* CNG	27	60
Sample 1	13	*MET* CNG	5	60
Sample 2	2	*MET* CNG	27	60
Sample 2	3	*MET* CNG	7	50
Sample 2	5	*MET* CNG	10	60
Sample 5	2	*MET* CNG	100	30
Sample 5	3	*MET* CNG	100	30
Sample 6 AC	1	*MET* CNG	36	162
Sample 6 AC	2	*MET* CNG	20	230
Sample 6 FC	1	*MET* CNG	81	171
Sample 6 FC	2	*MET* CNG	96	168
Sample 6 FC	8	*MET* CNG	100	37
Sample 7	1	*MET* CNG	0	30
Sample 8	1	*MET* CNG	20	30
Sample 8	3	*MET* CNG	0	30
Sample 3	1	*EGFR* CNG	100	30
Sample 4	1	*FGFR1* CNG	67	30
Sample 4	3	*FGFR1* CNG	70	60
Sample 9	1	*MYC* CNG	72	117
Sample 9	2	*MYC* CNG	63	60
Sample 9	6	*MYC* CNG	90	30
Sample 10	2	*MYC* CNG	100	79
Sample 10	6	*MYC* CNG	100	60

Table S3. Further details of the patients and samples included in the study. The methods used for the initial detection of fusions/CNGs in the same patient (in previous FFPE biopsies, cytological specimens or fluid supernatants) are indicated in the third column. After collecting the ascites or pleural effusions used to initiate the primary cultures, the number of positive cells present in the fluid was determined by FISH, when possible. The results are listed in the fourth column. If the fluid sample was small or the number of cells very limited, the entire sample was employed for initiation of the primary culture and FISH was performed at later passages. These cases are indicated by "ND".

Sample	Known Fusion/CNGs in previous material	Method used in previous material	% of Cells with fusion/CNG in fluid by FISH (passage 1)
1	*ALK* fusion / *MET* CNG	FISH	ND
2	*ALK* fusion / *MET* CNG	FISH	ND
3	*EGFR* CNG	FISH	100%
4	*FGFR1* CNG	NGS	90%
5	*MET* CNG	FISH	ND
6	*MET* CNG	NGS	81%
7	*MET* CNG	FISH	0%
8	*MET* CNG	FISH +IHC	20%
9	*MYC* CNG	NGS	ND
10	*MYC* CNG	NGS	ND
11	*ROS1* fusion	FISH	9%
12	*ROS1* fusion	FISH	76%

Table S4. Quality control (QC) parameters of the NGS analyses presented in Table 4.

Sample	Passage	Mean coverage depth	Percentage of targets coverage over 100 reads	Percentage of targets coverage under 20 reads	Results of NGS	
Sample 1	5	725	98.54	1.46	MET	10 copies
Sample 1	11	952	99.84	0.16	ND	ND
Sample 2	2	536	98.02	1.98	MET	NA
Sample 3	1	849	99.92	0.08	EGFR	5 copies
Sample 4	1	578	98.59	0.61	FGFR1	16 copies
Sample 5	2	913	99.95	0.05	ND	ND
Sample 6 FC	1	348	97.50	2.50	MET	>50 copies
Sample 7	1	410	98.51	0.51	ND	ND
Sample 8	1	840	99.73	0.11	ND	ND
Sample 9	2	1039	99.25	0.26	MYC	7 copies
Sample 10	2	141	70.39	10.58	MYC	>50 copies