

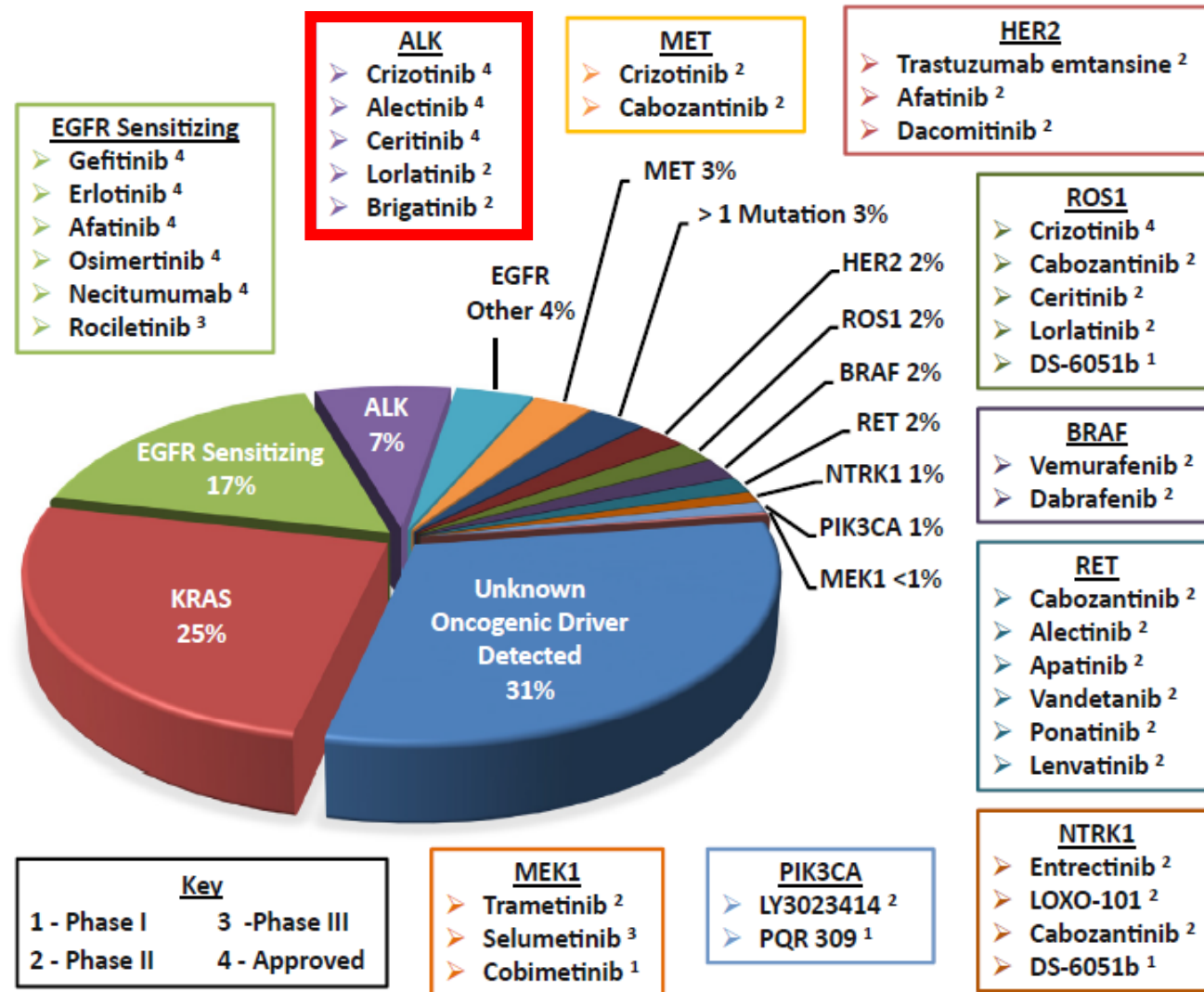
Application of Blood-based Liquid Biopsy in ALK-positive NSCLC

Cheol-Kyu Park

Department of Internal Medicine, Division of Lung Cancer Clinic

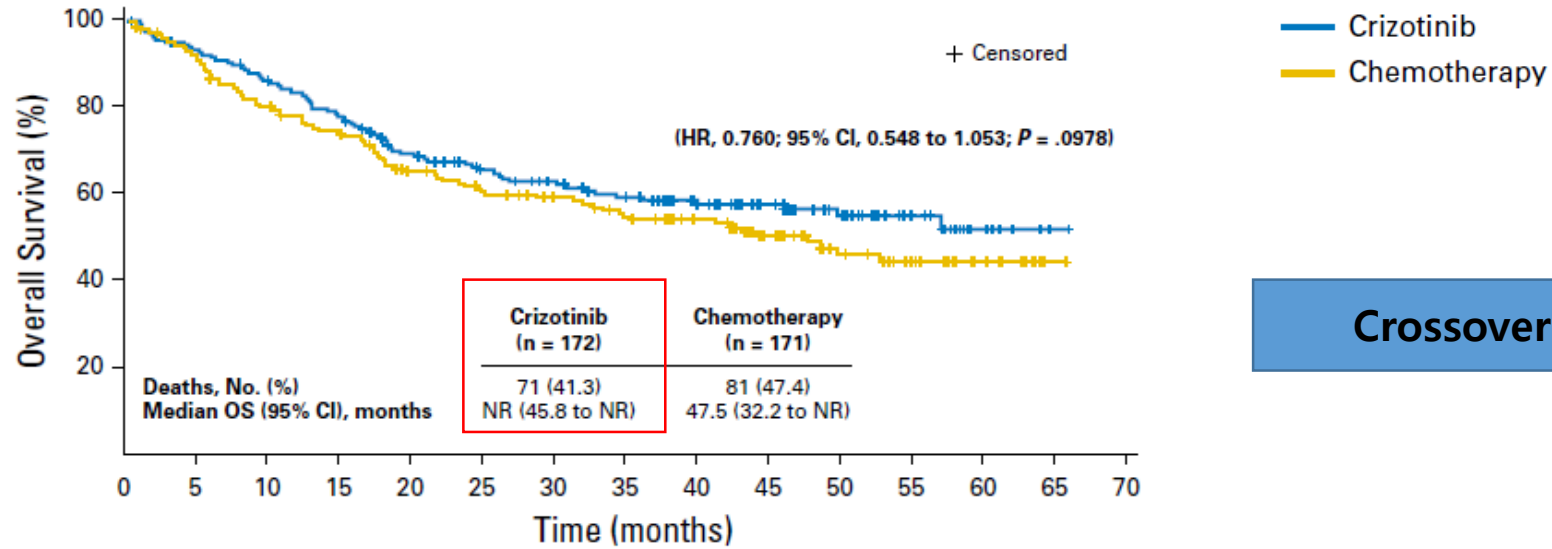
Chonnam National University Hwasun Hospital

ALK-positive NSCLC

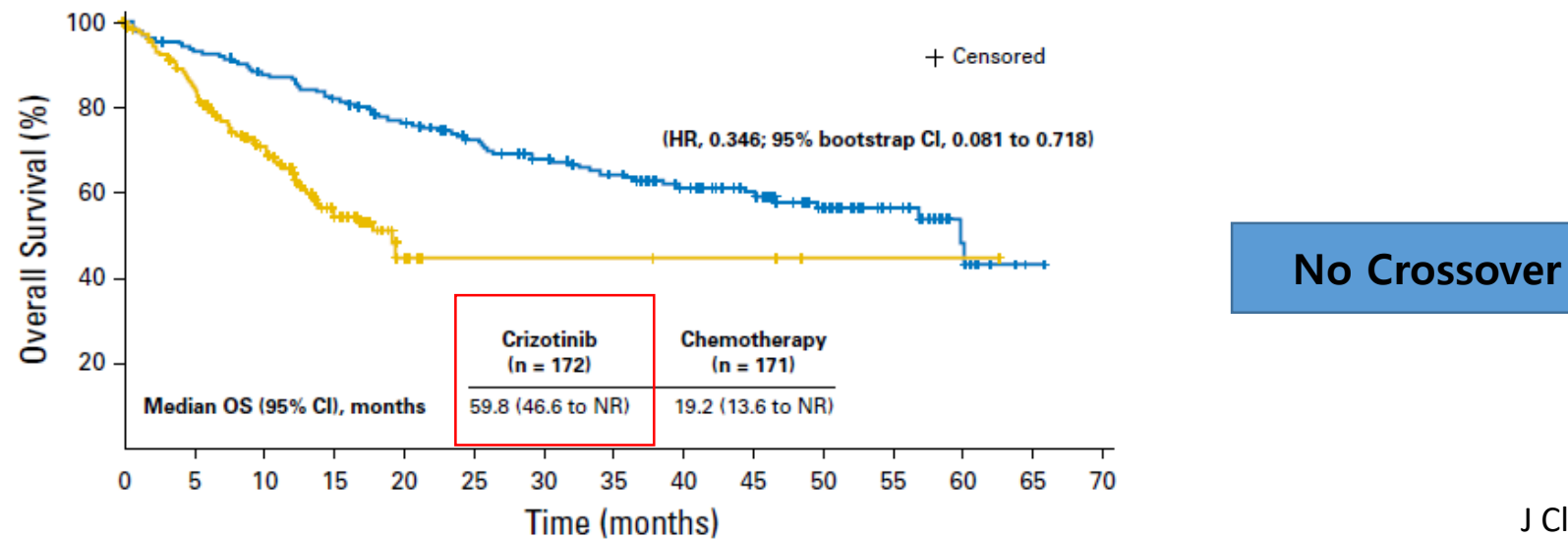


ALK-positive NSCLC : 1L Crizotinib (OS)

A



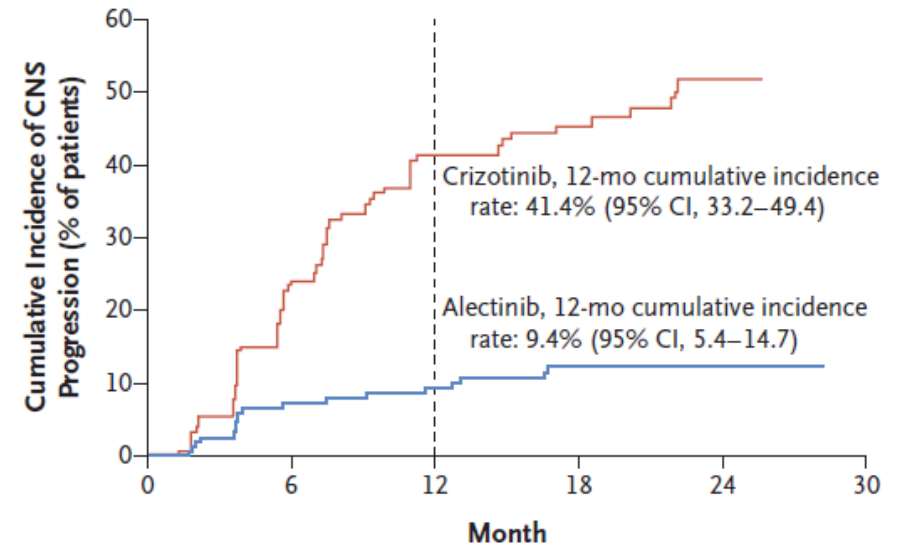
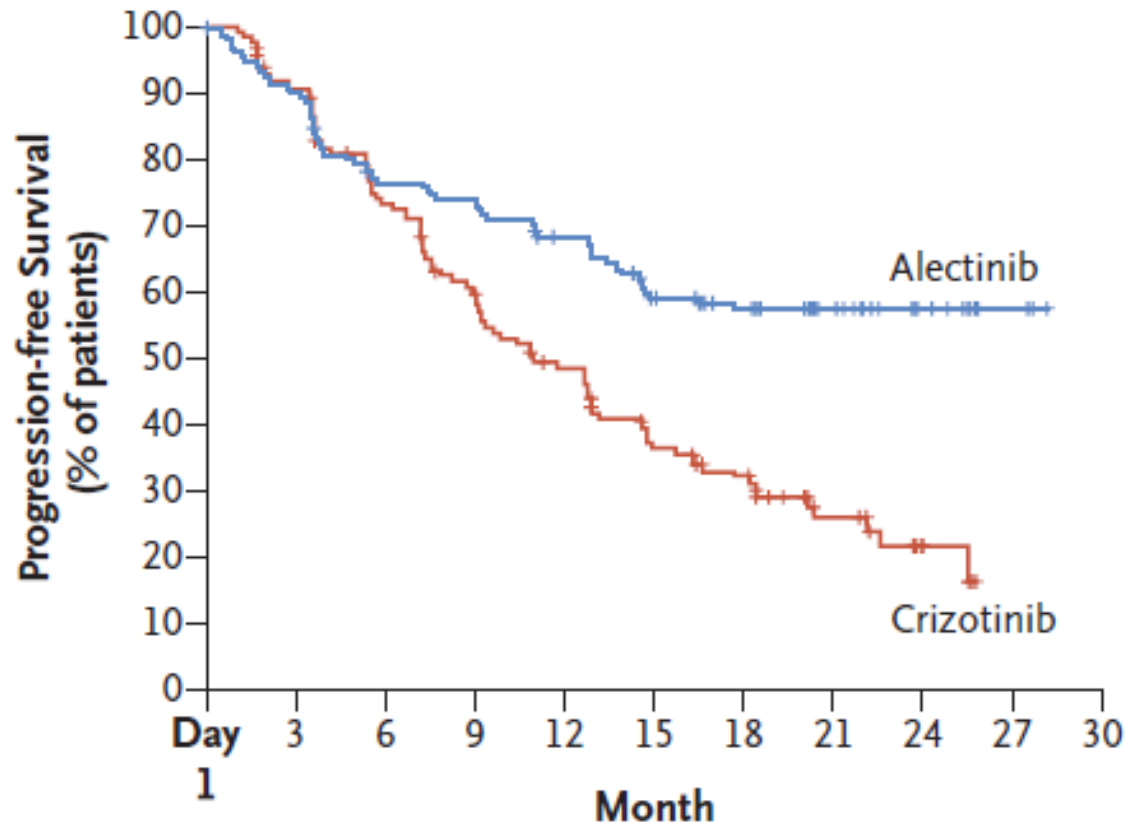
B



ALK-positive NSCLC : 1L Alectinib (PFS)

Hazard ratio for disease progression or death,
0.47 (95% CI, 0.34–0.65)
P<0.001 by log-rank test

~ 34 months



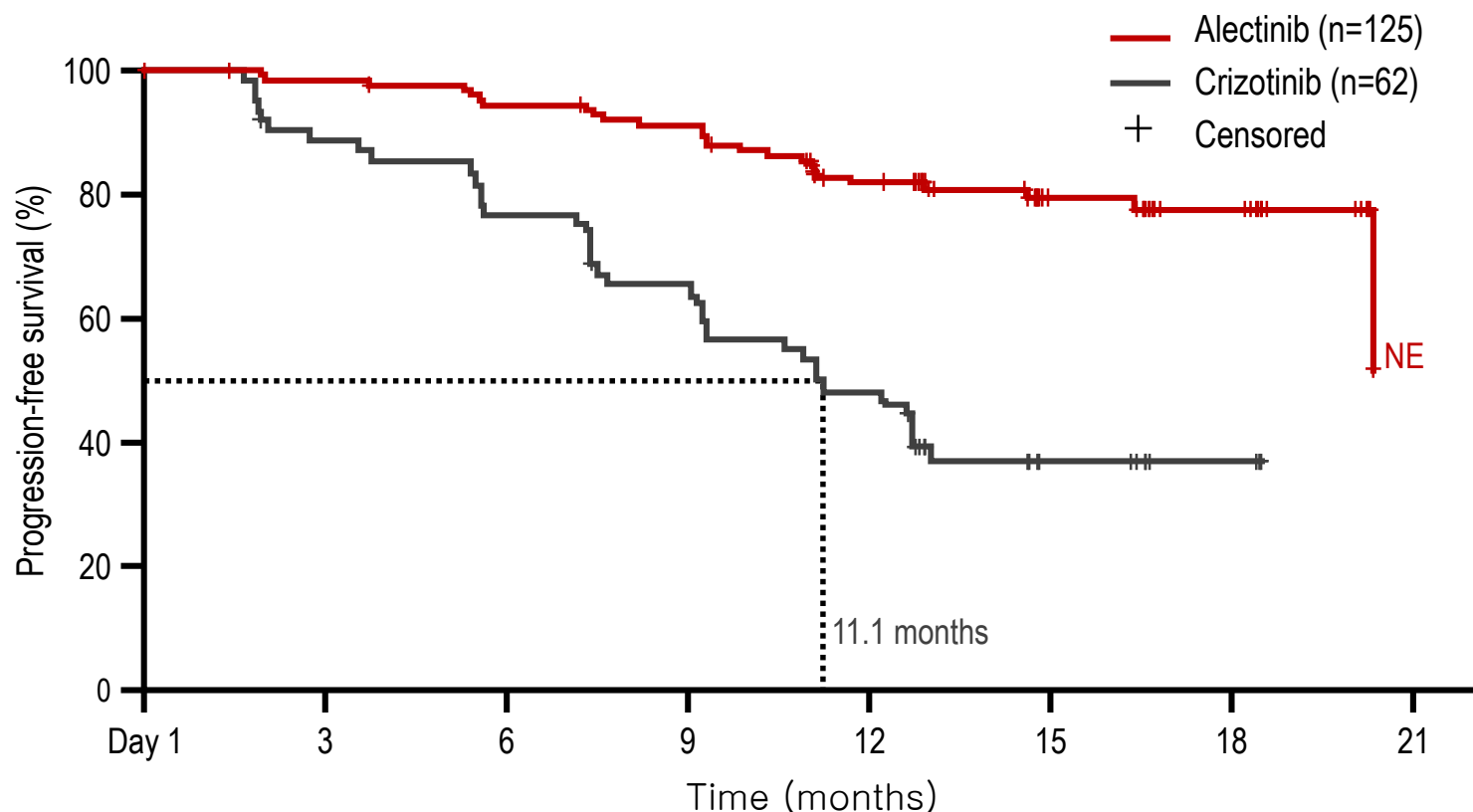
PRIMARY RESULTS OF **ALESIA**: A RANDOMISED, PHASE III, OPEN-LABEL STUDY OF ALECTINIB VERSUS CRIZOTINIB IN **ASIAN PATIENTS** WITH TREATMENT-NAIVE **ALK+** ADVANCED NSCLC

Zhou C,¹ Lu Y,² Kim S-W,³ Reungwetwattana T,⁴ Zhou J,⁵ Zhang Y,⁶ He J,⁷ Yang J-J,⁸ Cheng Y,⁹ Lee S-H,¹⁰ Bu L,¹¹ Xu T,¹¹ Yang L,¹¹ Wang C,¹¹ Liu T,¹¹ Morcos PN,¹² Mitry E,¹³ Zhang L¹⁴

¹Tongji University, Shanghai Pulmonary Hospital, Shanghai, China; ²West China Hospital, Sichuan University, Chengdu, China; ³Asan Medical Center, Seoul, South Korea; ⁴Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; ⁵The First Affiliated Hospital of College of Medicine, Zhejiang University, Hangzhou, China; ⁶Zhejiang Cancer Hospital, Hangzhou, China; ⁷The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China; ⁸Guangdong General Hospital, Guangzhou, China; ⁹Jilin Cancer Hospital, Changchun, China; ¹⁰Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea; ¹¹Roche Pharma Development, Shanghai, China; ¹²Roche Innovation Center, New York City, NY, USA; ¹³F. Hoffmann-La Roche Ltd., Basel, Switzerland (E. Mitry was an employee of F. Hoffmann-La Roche Ltd at the time of the study); ¹⁴Sun Yet-sen University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Guangzhou, China

ALESIA PROGRESSION-FREE SURVIVAL (INVESTIGATOR)

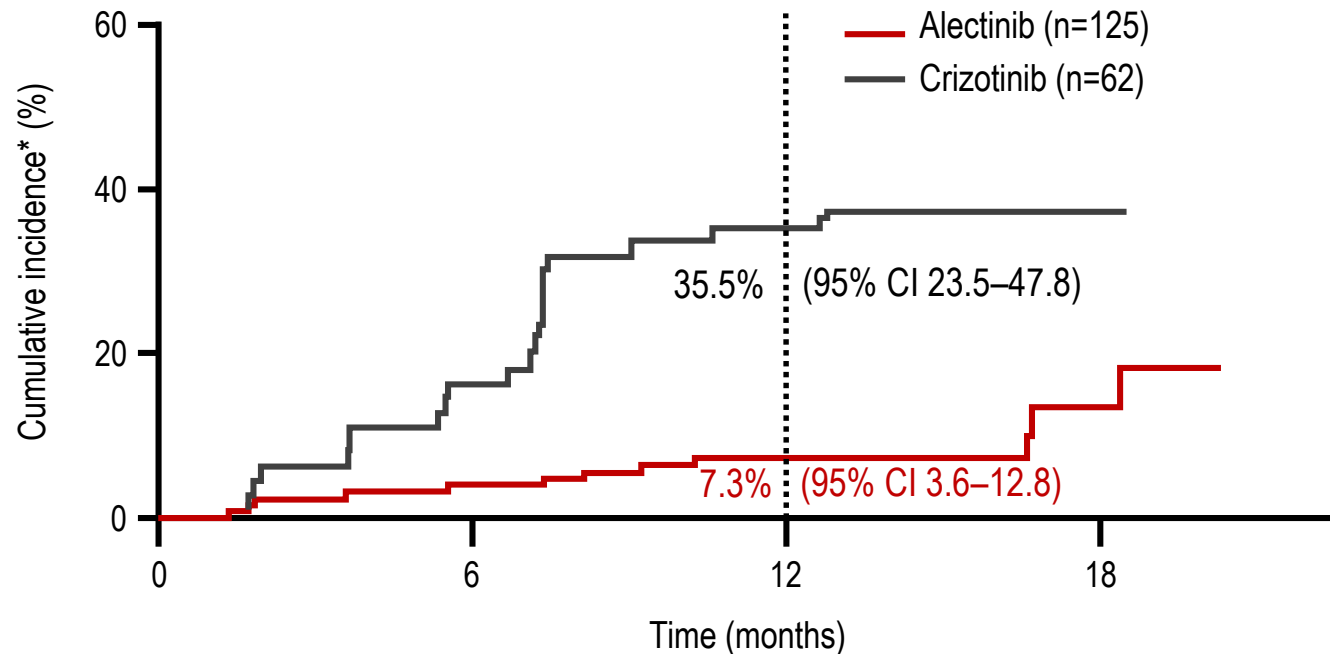
Primary endpoint



	Alectinib n=125	Crizotinib n=62
Patients with event, n (%)	26 (20.8)	37 (59.7)
Median PFS, months (95% CI)	NE (20.3–NE)	11.1 (9.1–13.0)
HR (95% CI) P-value (log-rank test)	0.22 (0.13–0.38) P<0.0001	

ALESIA TIME TO CNS PROGRESSION (IRC)

- A competing risk analysis with CNS progression, non-CNS progression and death as competing events was conducted; for each patient, only the first event was counted

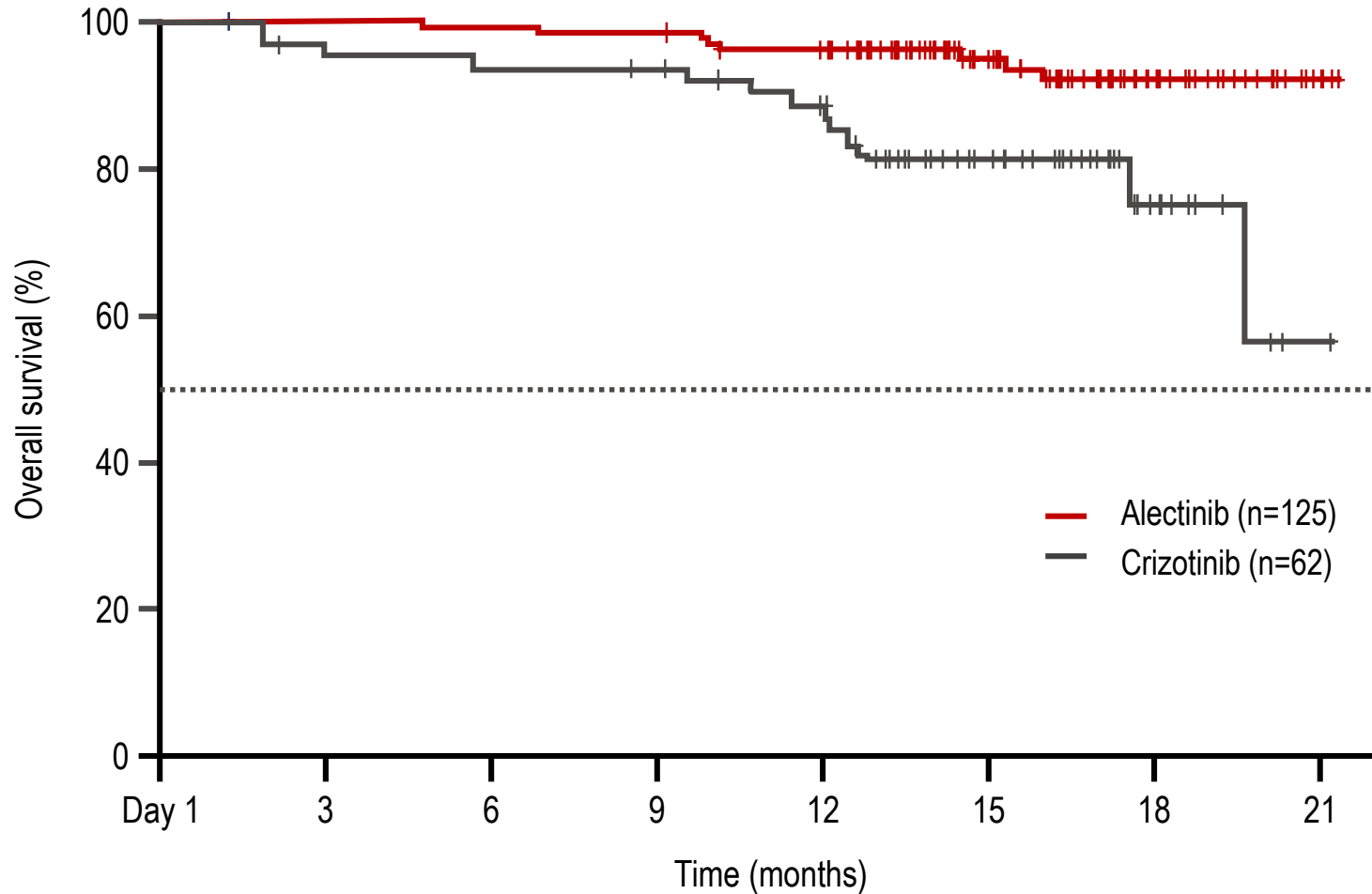


Cause-specific HR
(95% CI)
P-value (log-rank test)

0.14
(0.06–0.30)
P<0.0001

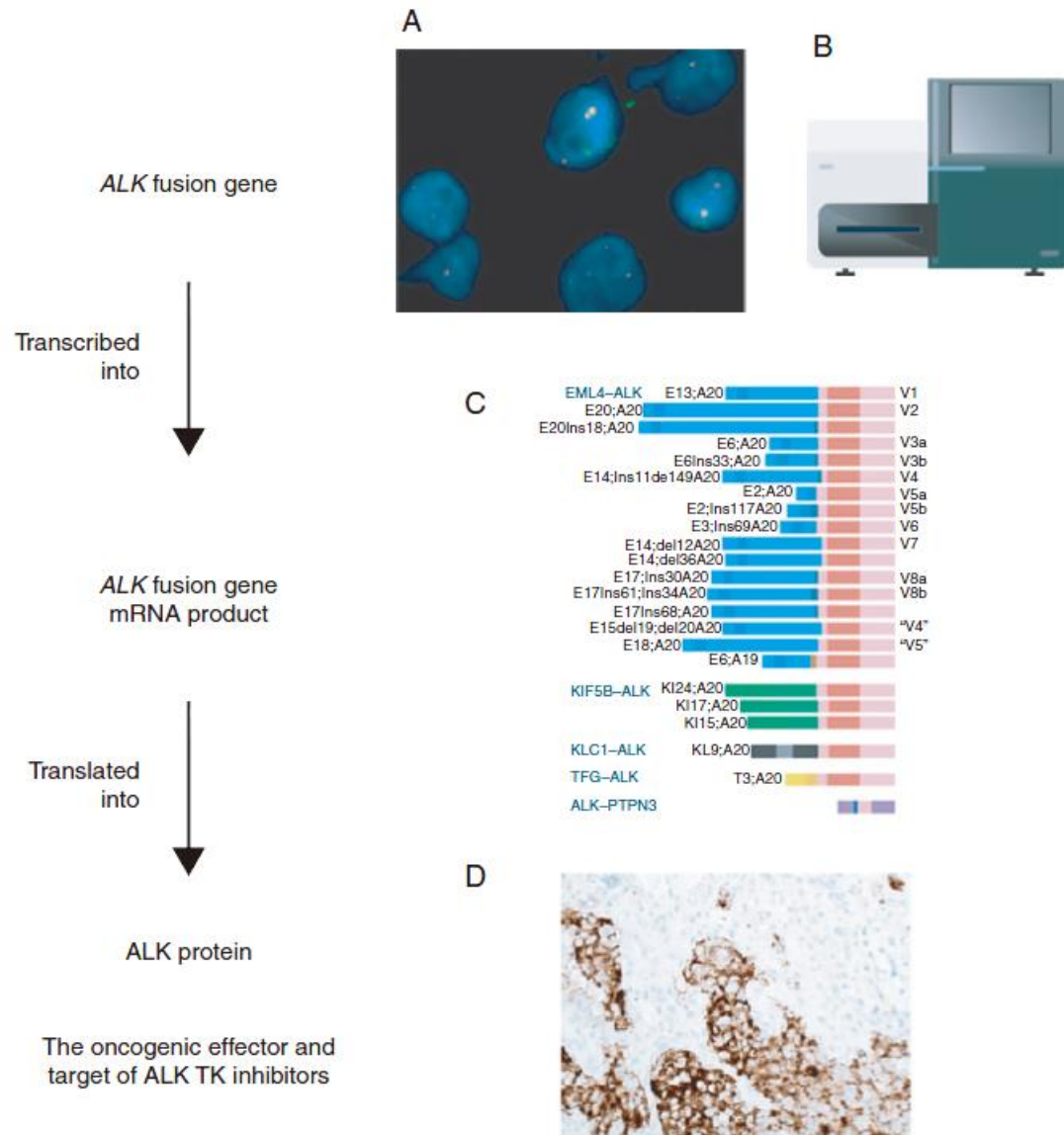
*Cumulative incidence of CNS progression without prior non-CNS progression or death

ALESIA OVERALL SURVIVAL



	Alectinib n=125	Crizotinib n=62
Patients with event, n (%)	8 (6.4)	13 (21.0)
Median, months (95% CI)	NE	NE (19.8–NE)
HR (95% CI)	0.28	(0.12–0.68)
P-value (log-rank test)		P=0.0027

Diagnosis of ALK-positive NSCLC



ALK fusion gene

Transcribed into

ALK fusion gene mRNA product

Translated into

ALK protein

The oncogenic effector and target of ALK TK inhibitors

FISH, PCR, NGS

Tissue, Liquid

PCR, NGS

Tissue, Liquid

IHC

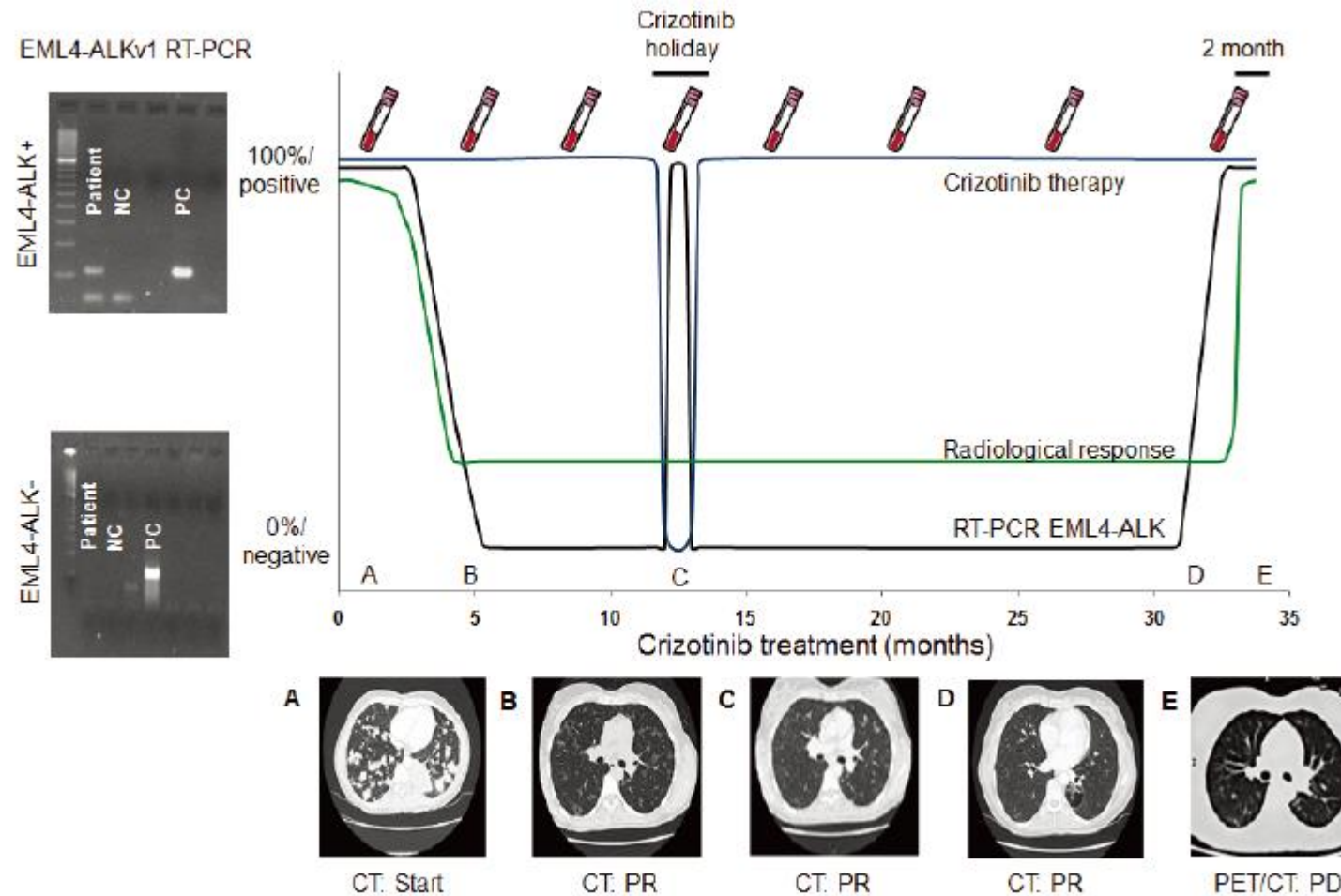
Tissue

Diagnostics in ALK-positive NSCLC

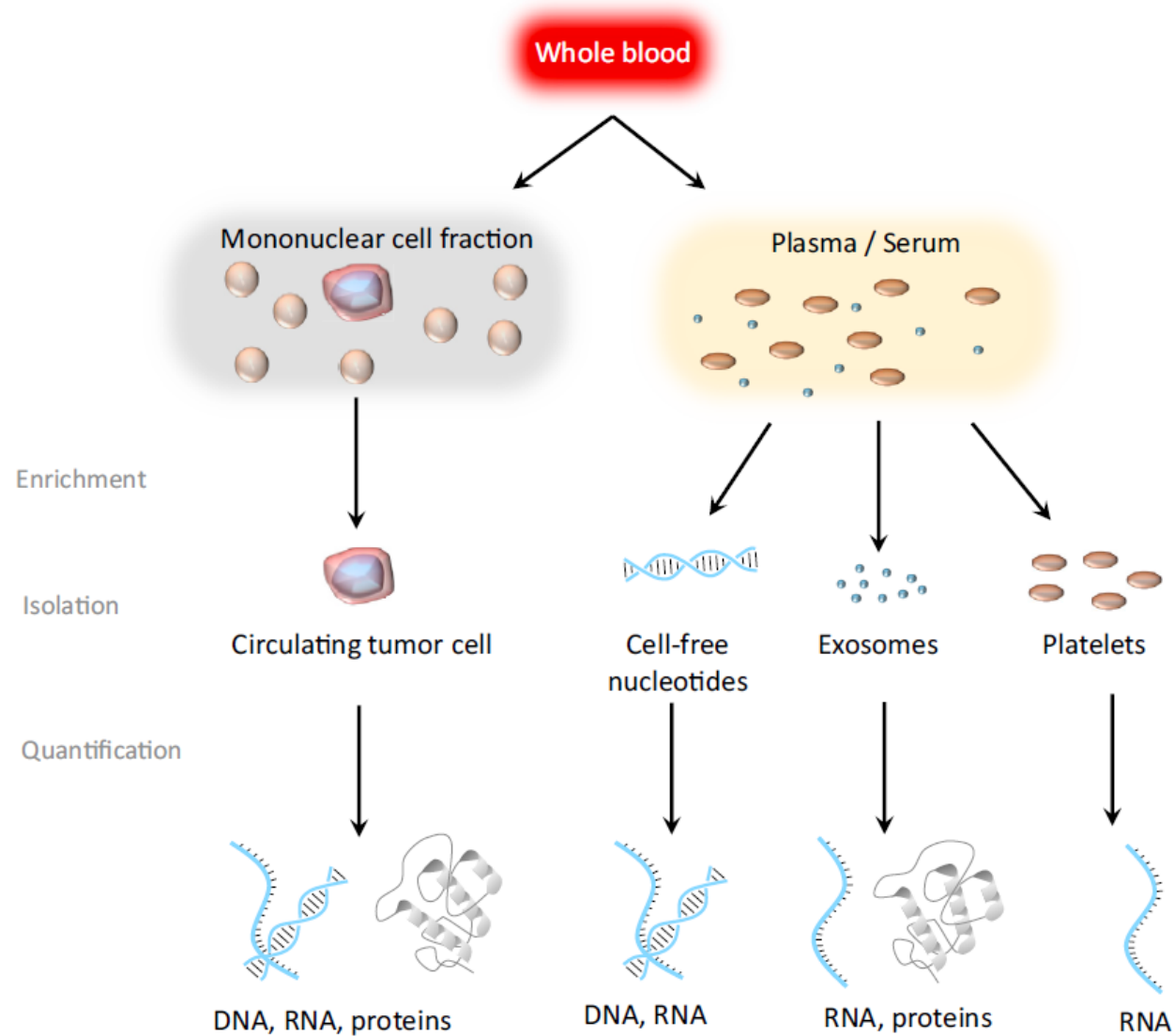
	FISH	IHC	RT-PCR	NGS
Current standard for <i>ALK</i> detection	Yes	No	No	No
Sensitivity	Break-apart signal can be subtle	High with detection enhancement	High	High
Detection of unknown variants	Yes	Yes	No	Yes
Labor intensive	Yes	No	No	No
Highly specialized training required	Yes	No	No	Yes
Simultaneous visualization of cell morphology	No	Yes	No	No
Widely used outside of specialized centers	No	Yes	No	No
Non-solid specimen	No	No	Yes	Yes
Speed, Accessibility	No	No	Yes	No
Facility, Cost	*	**	**	*****

Liquid Biopsy : Repeatability, Monitoring

- Longitudinal monitoring of Crizotinib response using Liquid biopsy



Blood-based Liquid Biopsy





Liquid Biopsy for Advanced Non-Small Cell Lung Cancer (NSCLC): A Statement Paper from the IASLC

- In contrast to EGFR, retrospective data suggests that **qPCR** probably is **not effective** enough for the detection of ALK rearrangements in **ctDNA** — efficacy in **platelet-derived** or **CTCs derived RNA** may be more promising but on a research level with a prospective cohort validation is still missing.
- While **ddPCR** has been shown to be far more **effective** at detecting ALK rearrangements in **ctDNA** compared to reverse transcriptase PCR, a prospective validation study is still missing.
- Conversely, **NGS** reached **acceptable** levels of sensitivity combined with optimal specificity in prospective cohorts—even though ALK rearrangement-specific data are not available.

Limitations in Application of Blood-based Liquid Biopsy in ALK-positive NSCLC

Which source of liquid biopsy ? **Which platform** for detection ?

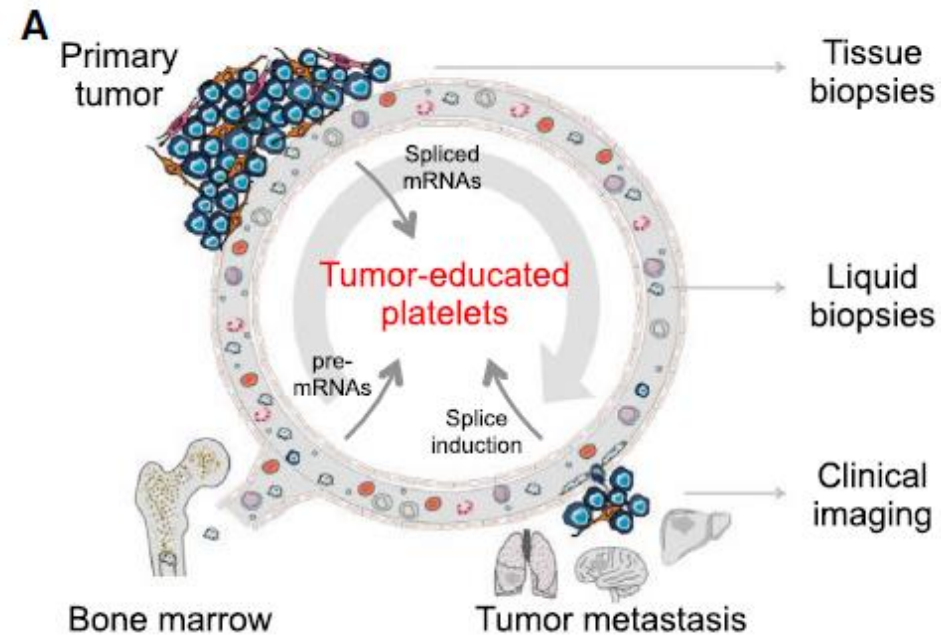
- **CTC** : not routine practice, required standardization in detection technique
- **cfDNA** : required extensively deep sequencing of genomic DNA for detection of the chromosomal break-point
- **EVs** : required standardization in isolation and analysis
- **ddPCR, BEAMing, NGS** : promising, but still barriers for use in daily practice

RNA analysis of Tumor-Educated Platelets (TEPs)

Article

Cancer Cell

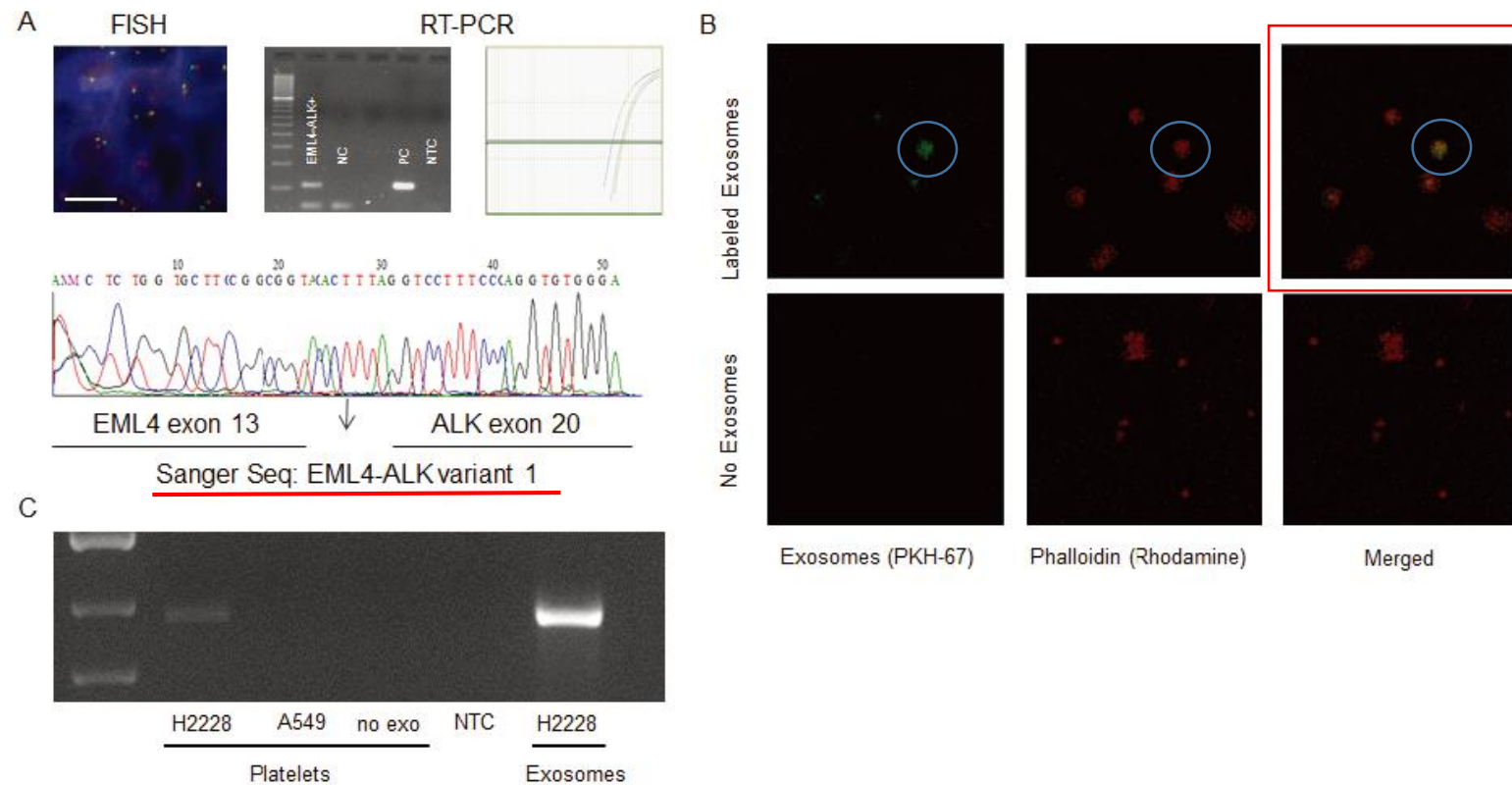
RNA-Seq of Tumor-Educated Platelets Enables Blood-Based Pan-Cancer, Multiclass, and Molecular Pathway Cancer Diagnostics



Detection of EML4-ALK using Platelets

Rearranged EML4-ALK fusion transcripts sequester in circulating blood platelets and enable blood-based crizotinib response monitoring in non-small-cell lung cancer

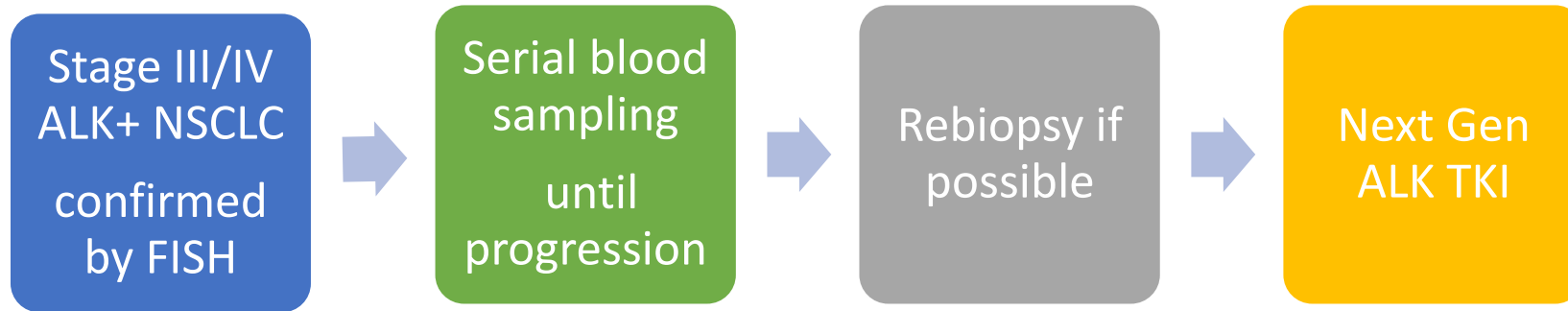
R. Jonas A. Nilsson^{1,2,3,*}, Niki Karachaliou^{4,*}, Jordi Berenguer¹, Ana Gimenez-Capitan⁵, Pepijn Schellen^{1,3}, Cristina Teixido⁵, Jihane Tannous⁶, Justine L. Kuiper⁷, Esther Drees¹, Magda Grabowska¹, Marte van Keulen⁶, Danielle A. M. Heideman⁸, Erik Thunnissen⁸, Anne-Marie C. Dingemans⁹, Santiago Viteri⁴, Bakhos A. Tannous⁶, Ana Drozdowskyj¹⁰, Rafael Rosell^{4,5,11,12,**}, Egbert F. Smit^{7,**} and Thomas Wurdinger^{1,3,6,**}



Objectives

- To investigate the **feasibility of blood-based liquid biopsy** using **plasma** and **platelets** for detection of ALK rearrangement.
: FISH vs **RT-PCR** with **commercial tissue-based kits**
- To evaluate the clinical/molecular characteristics of patients according to ALK positivity of liquid biopsy.
- To evaluate the predictive value of blood-based liquid biopsy for ALK inhibitor treatment.

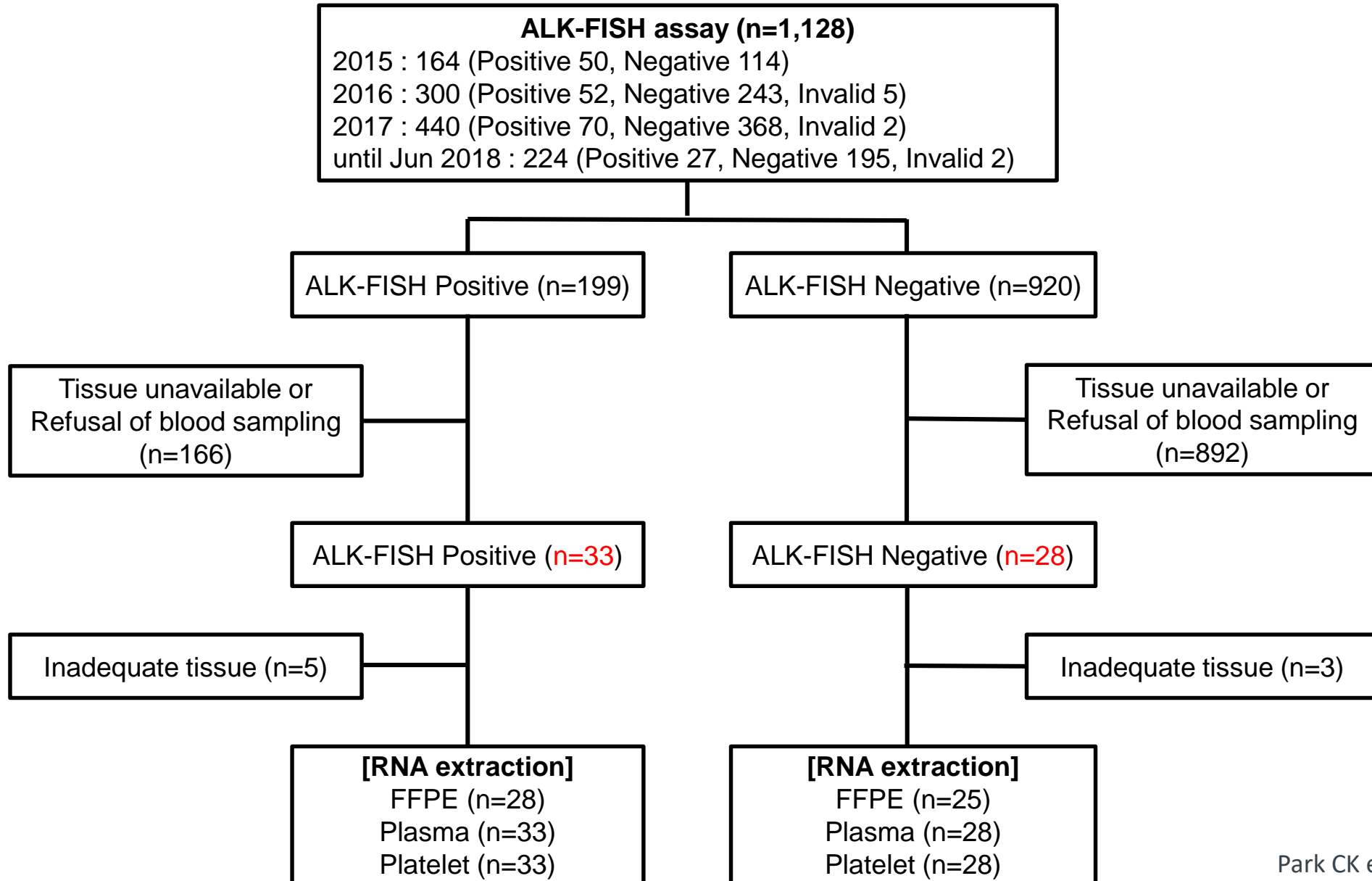
Study Design



Archival FFPE Blood (Plasma,Plt)	Sampling on visits 1M-1M-2M-3M....	Tumor tissue Blood (Plasma,Plt)	Sampling on visits 1M-1M-2M-3M....
RT-PCR	RT-PCR	NGS, RT-PCR	RT-PCR

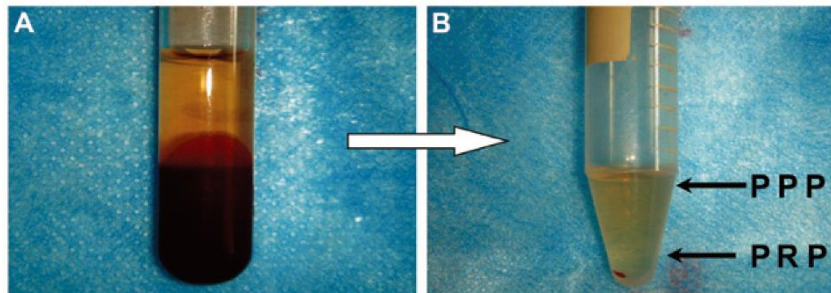
^aaccording to RECIST v1.1 or investigator's assessment

Patients



Methods – Sample collection

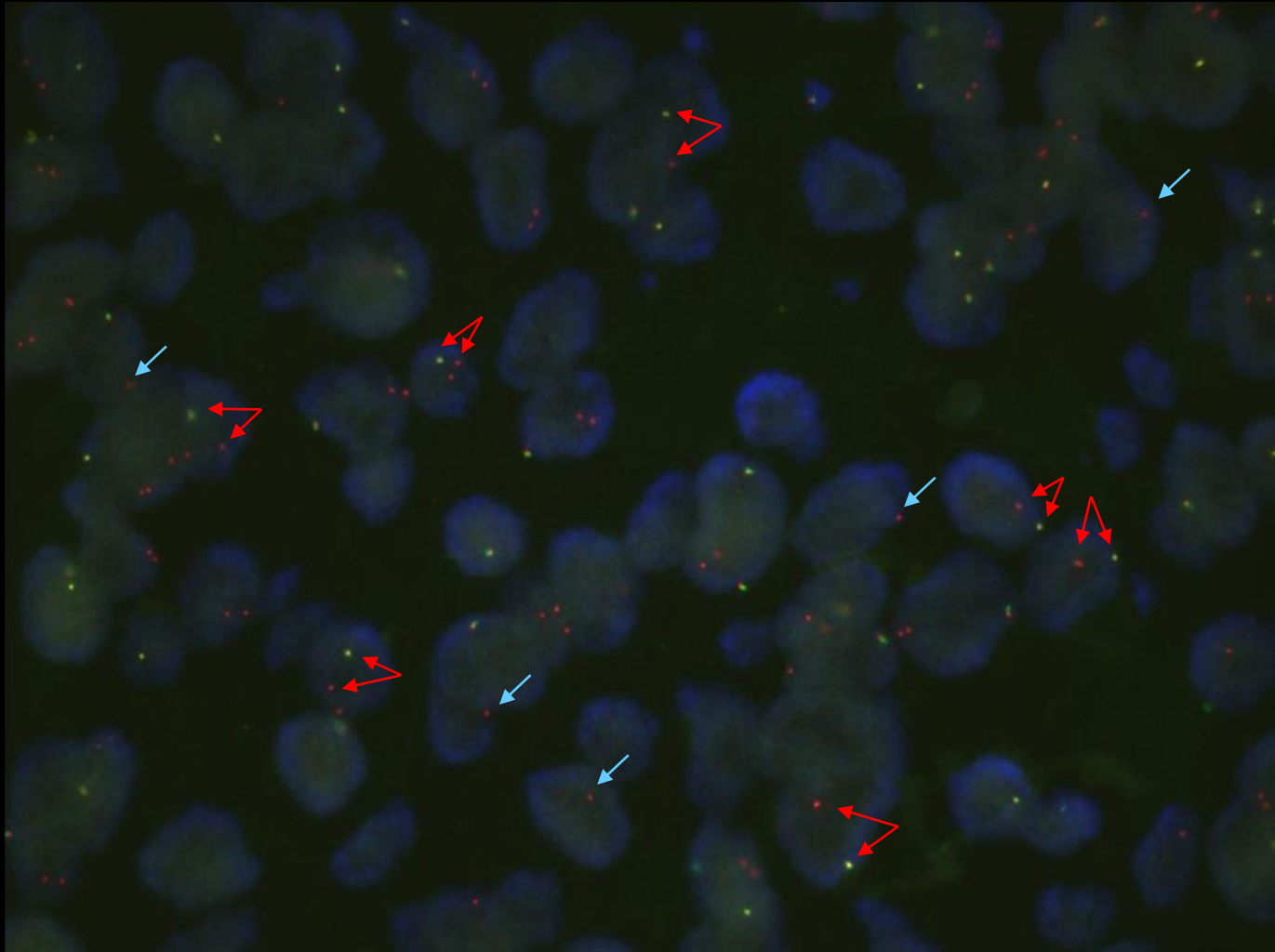
- **FFPE sections** from tumor tissues & **cell blocks** from cytology specimens (provided by **Biobank of Chonnam National University Hwasun Hospital**, a member of **Korea Biobank Network**)
- **Blood sampling & Plasma/Platelets isolation**
 - 10ml EDTA, Centrifugation within 2h from sampling



PPP : platelet-poor plasma
PRP : platelet-rich plasma

- Frozen in parallel at -80°C for further use (preserved at **Biobank of Chonnam National University Hwasun Hospital**) : + RNAlater solution
- Referral to central lab or In-house

Methods – FISH for FFPE



Methods – Preparation & Analysis

- RNA extraction & cDNA synthesis, RT-PCR (qPCR) assay



PANA qPCR™ EML4-ALK Detection Kit - Screening

PANA qPCR™ EML4-ALK Screening Kit

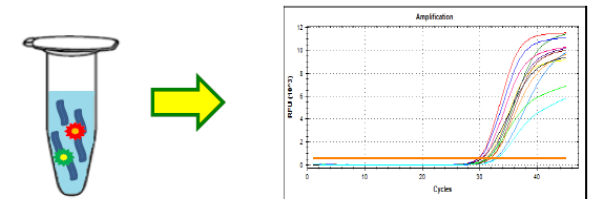
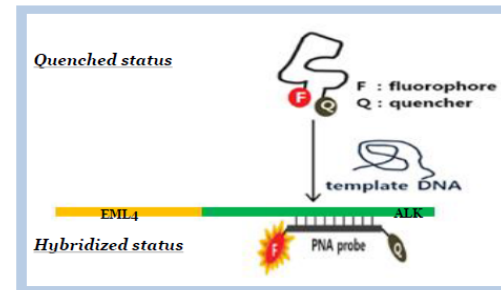
<Features>

Components	1 tube reaction (EML4-ALK fusion gene + Internal control)
Coverage	25 EML4-ALK fusion variants detectable*
Dye	ROX, HEX
Probe	Common Probe
Sensitivity	10 ² copy
Assessment	EML4-ALK positive or negative
Size	24 tests / kit

* Detectable variant list

	Variants	Targets	Sensitivity
1	V1	2	10 ² copy
2	V6	5	
3	V3a	3	
4	V3b	3	
5	V2	4	
6	V4	2	
7	V7	1	
8	Vnew	1	
9	V5a	1	
10	V5b	1	
11	V8a	1	
12	V8b	1	
Total		25	

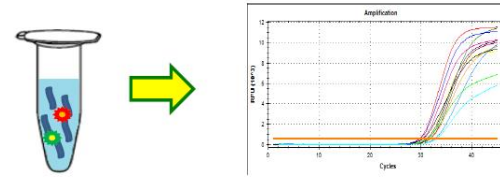
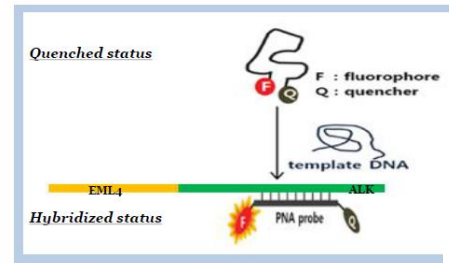
<Principle>



Using Common Probe → Detect 25 variants

❖ Detect mutation with PNA probe attached to the fluorophore and quencher

Methods – qPCR



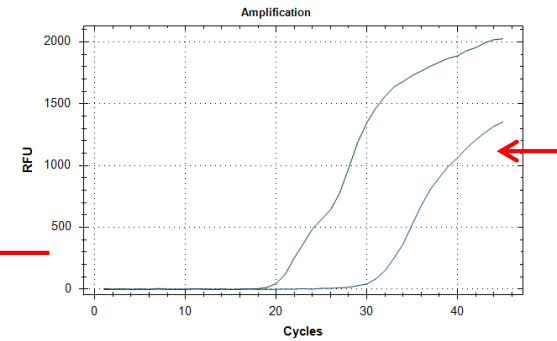
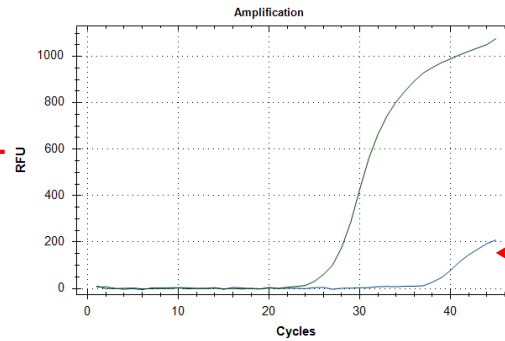
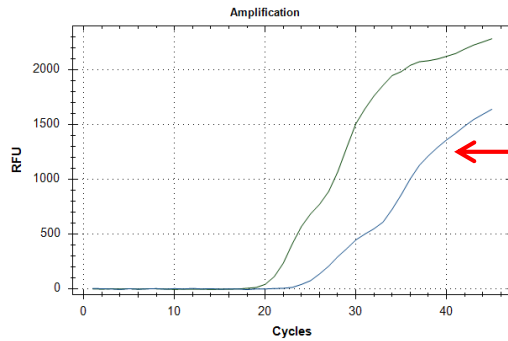
Using Common Probe → Detect 25 variants

FFPE

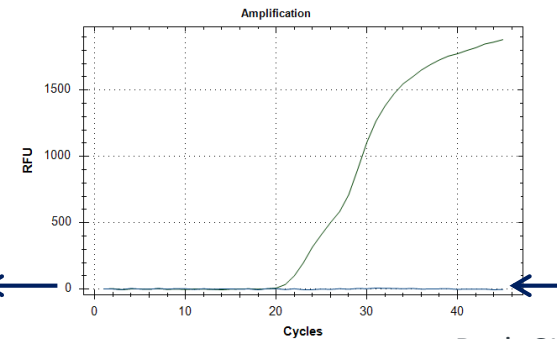
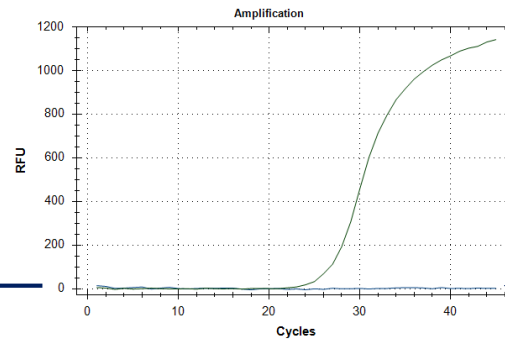
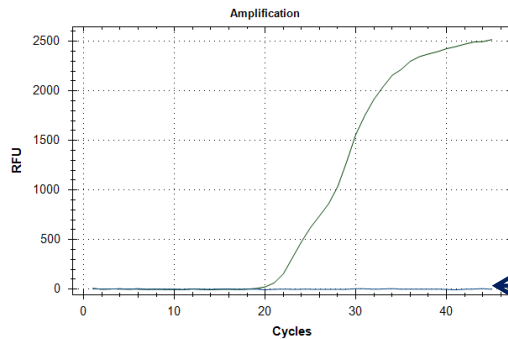
Plasma

Platelet

**Positive
(Ct <40)**



Negative



Results – Baseline characteristics (1)

Characteristics, n (%)	Total (n = 61)	FISH-positive (n = 33)	FISH-negative (n = 28)	p
Age, years, mean (SD)	63.7 (10.7)	61.3 (10.9)	66.5 (9.8)	0.076
Sex				0.178
Male	34 (55.7)	21 (63.6)	13 (46.4)	
Female	27 (44.3)	12 (36.4)	15 (53.6)	
Smoking				0.873
Ever-smoker	29 (47.5)	16 (48.5)	13 (46.4)	
Never-smoker	32 (52.5)	17 (51.5)	15 (53.6)	
Histology				0.416
ADC	59 (96.7)	31 (93.9)	28 (100.0)	
SQC	1 (1.6)	1 (3.0)	0 (0.0)	
ADSQC	1 (1.6)	1 (3.0)	0 (0.0)	
Differentiation				0.358
Well	9 (14.8)	3 (9.1)	6 (21.4)	
Moderate	13 (21.3)	9 (27.3)	4 (14.3)	
Poor	26 (42.6)	15 (45.5)	11 (39.3)	
Not evaluable	13 (21.3)	6 (18.2)	7 (25.0)	
EGFR mutation-positive	7 (11.4)	4 (12.1)	3 (10.7)	0.221
Stage at diagnosis				0.752
IIIA/IIIB	3 (4.9) / 2 (3.3)	1 (3.0) / 1 (3.0)	2 (7.1) / 1 (3.6)	
IV	56 (91.8)	31 (93.9)	25 (89.3)	
Intrathoracic	23 (41.1)	11 (35.5)	12 (48.0)	0.605
Extrathoracic (single)	13 (23.2)	9 (29.0)	4 (16.0)	0.217
Extrathoracic (multiple)	20 (35.7)	11 (35.5)	9 (36.0)	0.921

Results – Baseline characteristics (2)

Characteristics, n (%)	Total (n = 61)	FISH-positive (n = 33)	FISH-negative (n = 28)	p
Brain metastasis	19 (31.1)	13 (39.4)	6 (21.4)	0.131
Brain RT	12 (19.7)	10 (30.3)	2 (7.1)	0.023
Chemotherapy ----- Pemetrexed	36 (59.0)	22 (66.7)	14 (50.0)	0.187
Line, median (range)	1 (1–3)	1 (1–3)	1 (1–1)	0.267
Duration, months, median (range)	3.5 (0.0–42.7)	3.9 (0.0–42.7)	3.5 (0.0–13.4)	0.141
PFS, months, median (95% CI)	3.9 (3.1–4.8)	3.9 (3.3–4.6)	1.5 (0.0–5.5)	0.182
Overall response rate	9/36 (25.0)	4 (18.2)	5 (35.7)	0.267
Disease control rate	26/36 (72.2)	18 (81.8)	8 (57.1)	0.140

Results – Detection of EML4-ALK using RT-PCR

RT-PCR, n (%)	FISH-positive (n = 33)	FISH-negative (n = 28)
FFPE		
Positive	18 (54.5)	3 (10.7)
Negative	10 (30.3)	22 (78.6)
Inadequate	5 (15.2)	3 (10.7)
Accuracy	40/53 (75.5)	
Plasma		
Positive	21 (63.6)	1 (3.6)
Negative	12 (36.4)	27 (96.4)
Accuracy	48/61 (78.7)	
Platelet		
Positive	23 (69.7)	2 (7.1)
Negative	10 (30.3)	26 (92.9)
Accuracy	49/61 (80.3)	
Liquid biopsy		
Positive ^a	26 (78.8)	3 (10.7)
Negative ^b	7 (21.2)	25 (89.3)
Accuracy	51/61 (83.6)	

^aplasma or platelet, ^bplasma and platelet

Results – Molecular/Clinical characteristics (1)

Characteristics, n (%)	Total (N = 33)	Plasma		p	Platelet		p
		+ (N = 21)	- (N = 12)		+ (N = 23)	- (N = 10)	
FISH-positive proportion, %, median (range)	15.0 (15.0–80.0)	20.0 (15.0–70.0)	15.0 (15.0–80.0)	0.082	20.0 (15.0–80.0)	15.0 (15.0–35.0)	0.084
Total RNA, ng/μL, mean (SD)							
FFPE/plasma/platelets		93.45 (124.10) / 2.26 (0.63) / 2.71 (1.08)					
Liquid		2.34 (0.61)	2.04 (0.66)	0.252	2.74 (1.21)	2.55 (1.10)	0.784
Time point at sampling							
Before initiation of systemic treatment	3 (9.1)	0 (0.0)	3 (25.0)	0.040	0 (0.0)	3 (30.0)	0.022
After initiation of systemic treatment	30 (90.9)	21 (100.0)	9 (75.0)	-	23 (100.0)	7 (70.0)	-
After CTx and before ALK TKI	13 (43.3)	9 (42.9)	4 (44.4)	0.936	12 (52.2)	1 (14.3)	0.077
After ALK TKI	17 (56.7)	12 (57.1)	5 (55.6)	-	11 (47.8)	6 (85.7)	-
Interval from diagnosis to sampling, months, median (range)	11.7 (5.9–21.7)	11.7 (3.7–120.0)	10.8 (0.0–73.5)	0.681	11.7 (1.6–120.0)	9.8 (0.0–57.8)	0.411
< 6 months, n (%)	8 (24.2)	3 (14.3)	5 (41.7)	0.106	3 (13.0)	5 (50.0)	0.036
≥ 6 months, n (%)	25 (75.8)	18 (85.7)	7 (58.3)	-	20 (87.0)	5 (50.0)	-
Treatment before crizotinib	30 (90.9)	21 (100.0)	9 (75.0)	0.040	23 (100.0)	7 (70.0)	0.022
Operation	6 (18.2)	4 (19.0)	2 (16.7)	1.000	5 (21.7)	1 (10.0)	0.640
RT	4 (12.1)	1 (4.8)	3 (25.0)	0.125	3 (13.0)	1 (10.0)	1.000
Chemotherapy	24 (72.7)	19 (90.5)	5 (41.7)	0.005	18 (78.3)	6 (60.0)	0.400
EGFR TKI	5 (15.2)	4 (19.0)	1 (8.3)	0.630	5 (21.7)	0 (0.0)	0.291
Prior Tx except operation	29 (87.9)	21 (100.0)	8 (66.7)	0.012	23 (100.0)	6 (60.0)	0.005
Treatment after crizotinib	16 (48.5)	10 (47.6)	6 (50.0)	0.476	11 (47.8)	5 (50.0)	0.107
Chemotherapy	8 (24.2)	5 (23.8)	3 (25.0)		4 (17.4)	4 (40.0)	
ALK TKI	11 (33.3)	8 (38.1)	3 (25.0)		9 (39.1)	2 (20.0)	
EGFR TKI	2 (6.1)	2 (9.5)	0 (0.0)		2 (8.7)	0 (0.0)	
ICI	3 (9.1)	3 (14.3)	0 (0.0)		2 (8.7)	1 (10.0)	
BSC	1 (3.0)	0 (0.0)	1 (8.3)		0 (0.0)	1 (10.0)	

Results – Molecular/Clinical characteristics (2)

Characteristics, n (%)	Total (N = 33)	Plasma		<i>p</i>	Platelet		<i>p</i>
		+ (N = 21)	- (N = 12)		+ (N = 23)	- (N = 10)	
ALK TKI – Crizotinib	26 (78.8)	16 (76.2)	10 (83.3)	1.000	17 (73.9)	9 (90.0)	0.397
Line, median (range)	2 (1–6)	2 (1–6)	2 (1–3)	0.066	2 (1–6)	2 (1–3)	0.924
Duration, months, median (range)	5.6 (0.3–39.6)	5.3 (0.3–39.6)	4.0 (0.6–39.5)	1.000	7.2 (0.7–39.6)	1.5 (0.3–39.5)	0.090
PFS, months, median (95% CI)	5.2 (2.5–8.0)	5.4 (2.8–8.0)	4.0 (0.0–10.0)	0.713	5.7 (0.0–16.7)	1.7 (0.5–3.0)	0.028
Best response							
PR	13 (50.0)	9 (56.3)	4 (40.0)		12 (70.6)	1 (11.1)	
SD	6 (23.1)	3 (18.8)	3 (30.0)		3 (17.6)	3 (33.3)	
PD	6 (23.1)	4 (25.0)	2 (20.0)		2 (8.7)	4 (44.4)	
NE	1 (3.8)	0 (0.0)	1 (10.0)		0 (0.0)	1 (11.1)	
Overall response rate	13/26 (50.0)	9/16 (56.3)	4/10 (40.0)	0.420	12/17 (70.6)	1/9 (11.1)	0.011
Disease control rate	19/26 (73.1)	12/16 (75.0)	7/10 (70.0)	1.000	15/17 (88.2)	4/9 (44.4)	0.028

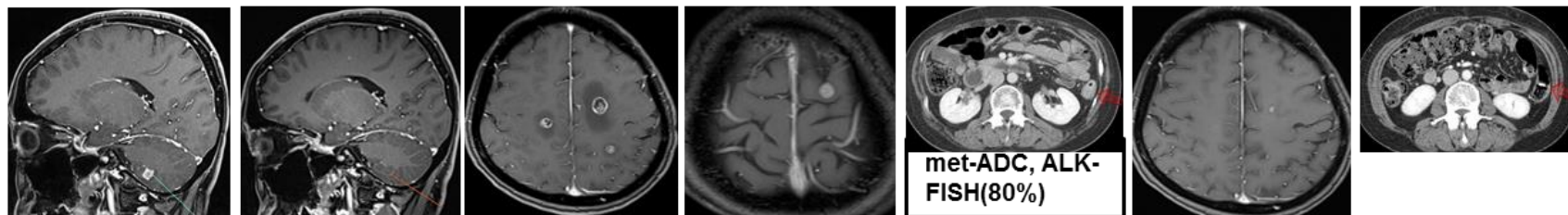
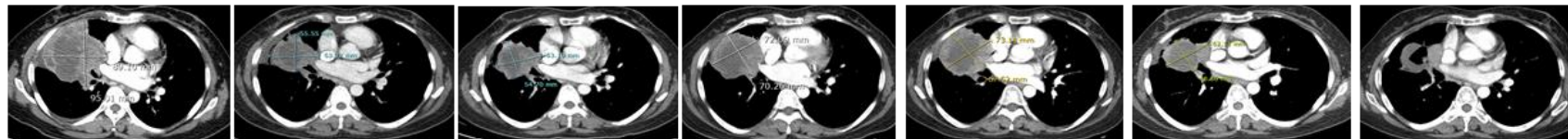
Serial monitoring during ALK inhibitor treatment (Case #1-3)

- Serial blood sample collection : **n=12** (ALK-TKI Tx : n=26)
 - 4 patients : initially positive
 - 5 patients : positive conversion
 - 3 patients : continuously negative

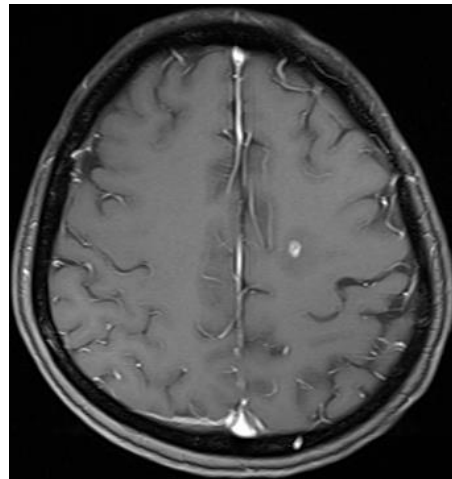
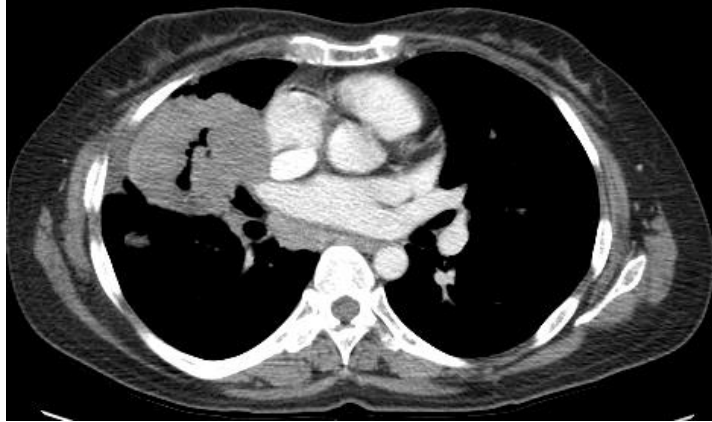
Case #1 : 52/F, ADC, RUL, IVB (M1c : Rt.pleura, both adrenal, brain)

EML4-ALK RT-PCR ■ positive ■ negative

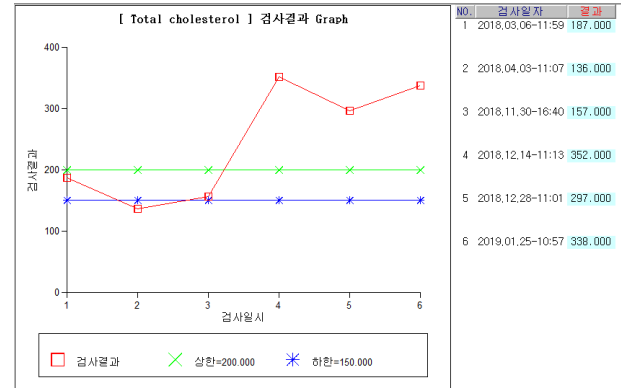
		2017-07-27		2017-09-29		2017-12-01		2018-01-26		2018-05-08		2018-06-05		2018-07-03	
Pls	Pit	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	FAM	NA	30.91	NA	30.88	NA	30.16	36.02	34.62	NA	33.63	NA	NA	NA	NA
Ct	IC	25.13	21.45	23.40	21.20	24.37	21.28	35.20	29.81	23.30	20.01	24.43	22.78	25.40	21.99
Treatment		Crizotinib								Alectinib					
		GKS						GKS		Colon OP, GKS					
Chest CT		Baseline		PR		SD		SD		PD		PR		SD	



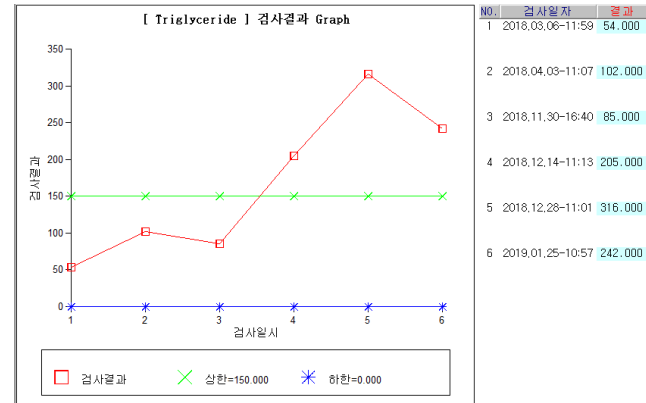
Case #1 : 52/F, ADC, RUL, IVB (M1c : Rt.pleura, both adrenal, brain)



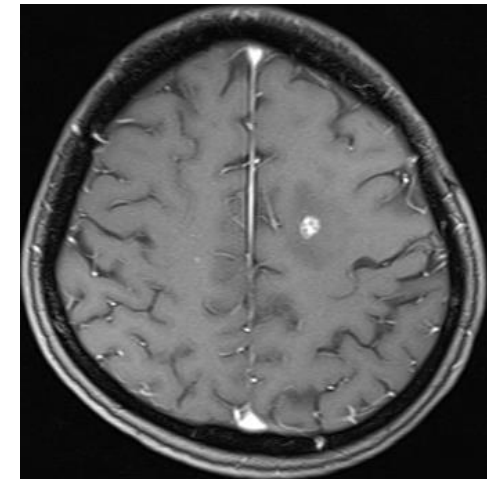
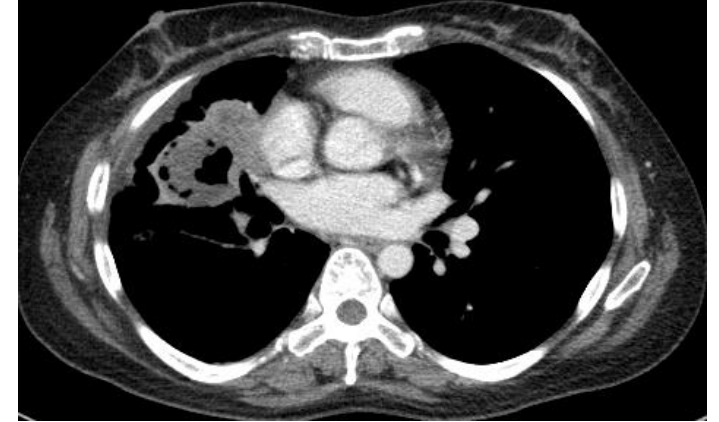
2018-11-05



Lorlatinib



2018-11-30

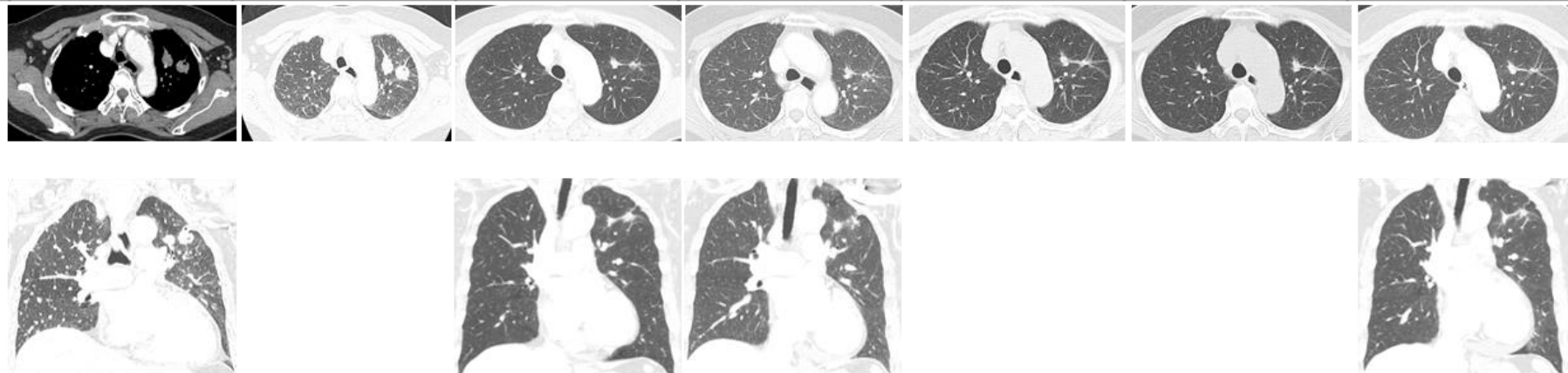


2019-01-18

Case #2 : 67/F, ADC, LUL, IVA (M1a : lung to lung)

EML4-ALK RT-PCR ■ positive ■ negative

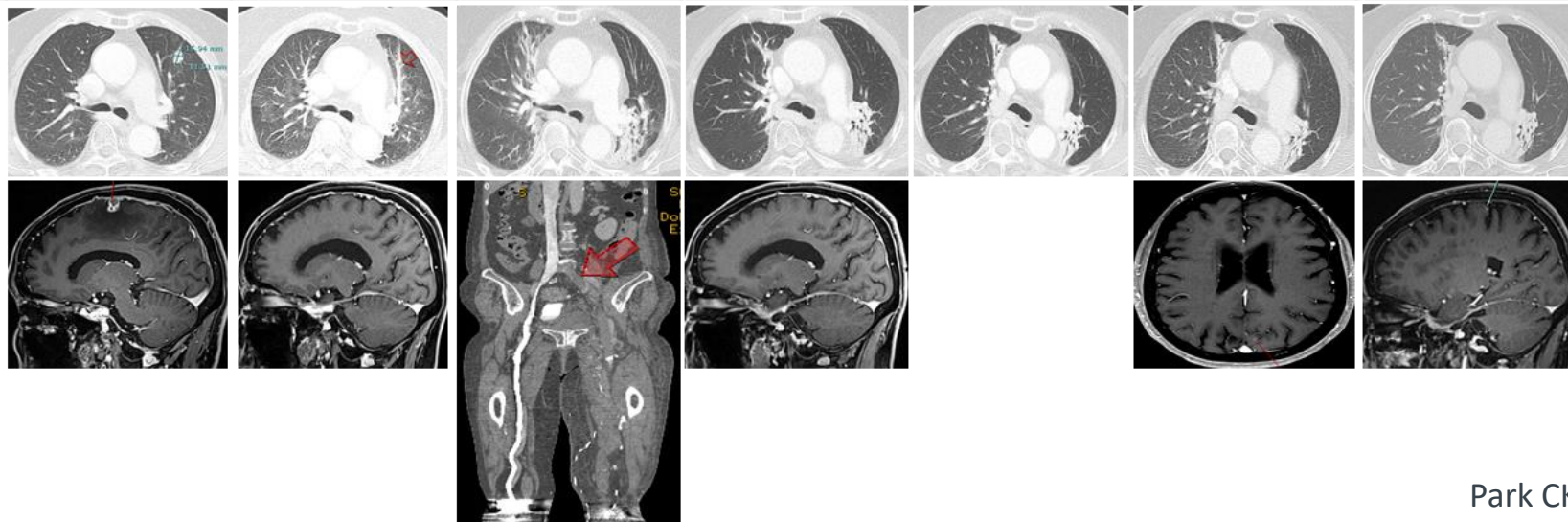
		2017-08-03		2017-08-30		2017-09-27		2017-11-29		2018-01-24		2018-05-16		2018-07-18	
Pls	Plt	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	FAM	NA	31.38	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ct	IC	23.20	21.09	27.07	20.16	25.65	20.87	27.25	20.51	27.86	20.91	27.10	19.98	28.33	21.07
	Treatment	Crizotinib													
Chest CT		Baseline	-	PR	SD	SD	SD	SD	SD	SD	SD	SD	SD	SD	SD



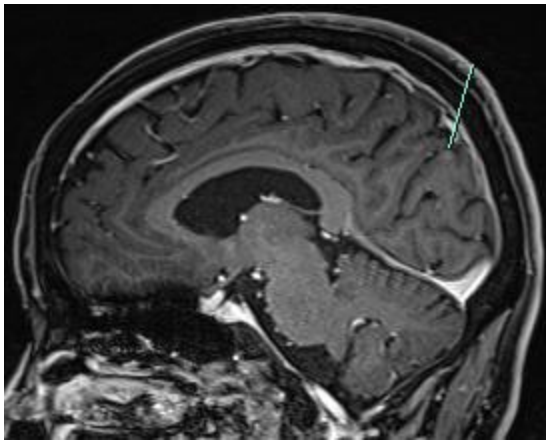
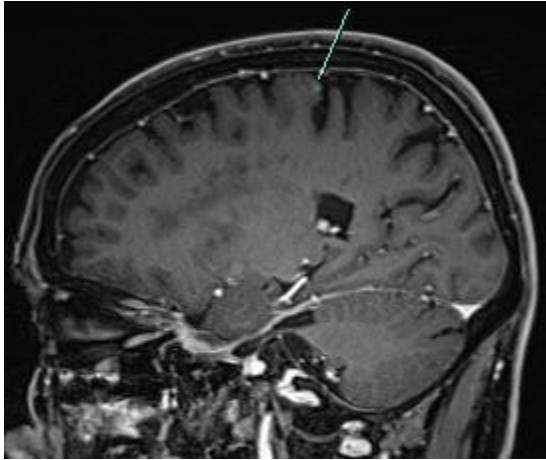
Case #3 : 78/F, ADC, LUL, IIBB → IVB (M1c : brain, multiple)

EML4-ALK RT-PCR ■ positive ■ negative

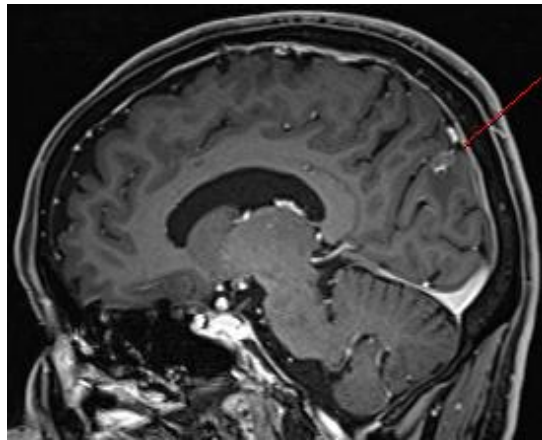
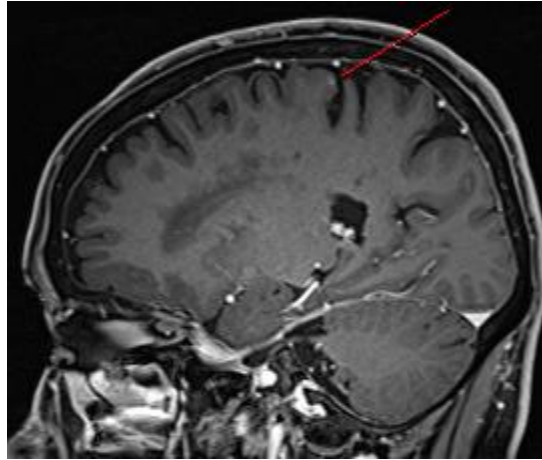
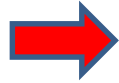
		2017-05-31		2017-07-28		2017-09-19		2017-12-06		2018-01-30		2018-04-17		2018-06-14	
Pls	Plt	 		 		 		 		 		 		 	
	FAM	NA	NA	NA	NA	NA	NA	NA	NA	33.51	38.34	NA	32.71	30.47	NA
Ct	IC	27.41	22.36	26.16	21.84	24.66	21.12	26.14	21.04	34.07	31.41	26.08	23.32	26.78	22.70
	Treatment	Crizotinib													
		GKS				PTA		PTA							
Chest CT		Baseline		PR		SD		SD		SD		SD		SD	



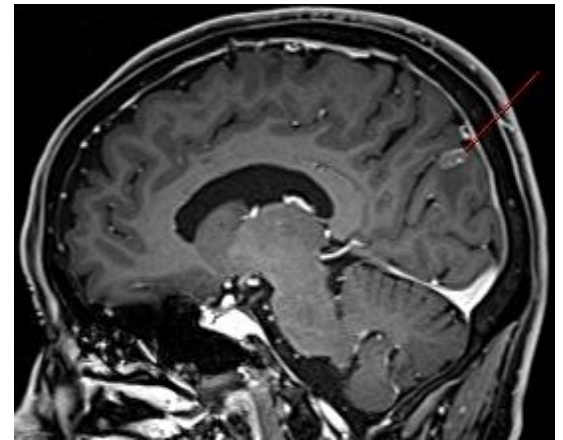
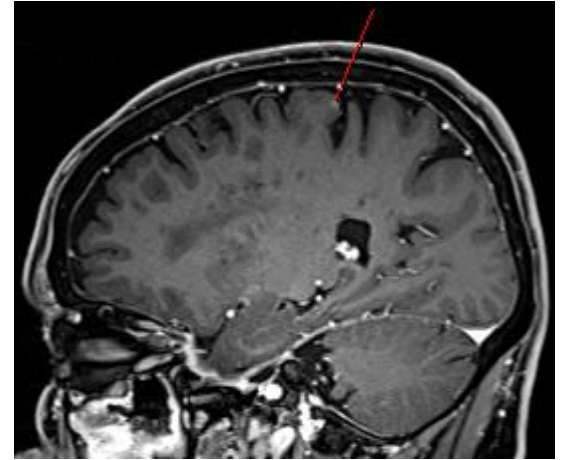
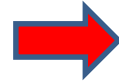
Case #3 : 78/F, ADC, LUL, IIIB → IVB (M1c : brain, multiple)



2018-07-18



2018-10-22



2018-12-19

Conclusions

- **Plasma and platelets** are a valuable and complementary sources for liquid biopsy in **detection of ALK rearrangement**, and showed favorable sensitivity despite using **tissue-based RT-PCR kit**.
- Plasma and platelets could play a **supplementary** role in **diagnosis of ALK-positive NSCLC**, particularly during the later period after diagnosis.
- **Platelets** may be useful for **predicting the treatment outcome** of ALK inhibitors.

Limitations

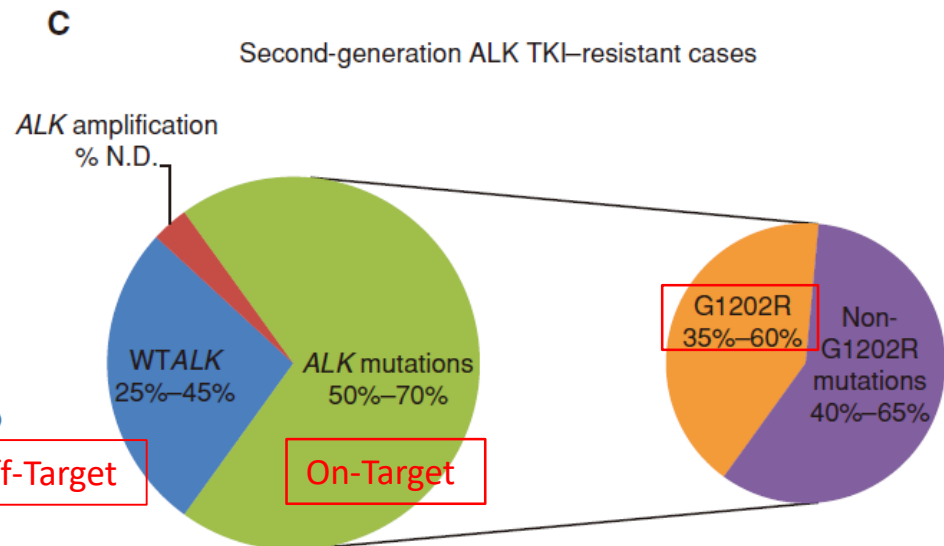
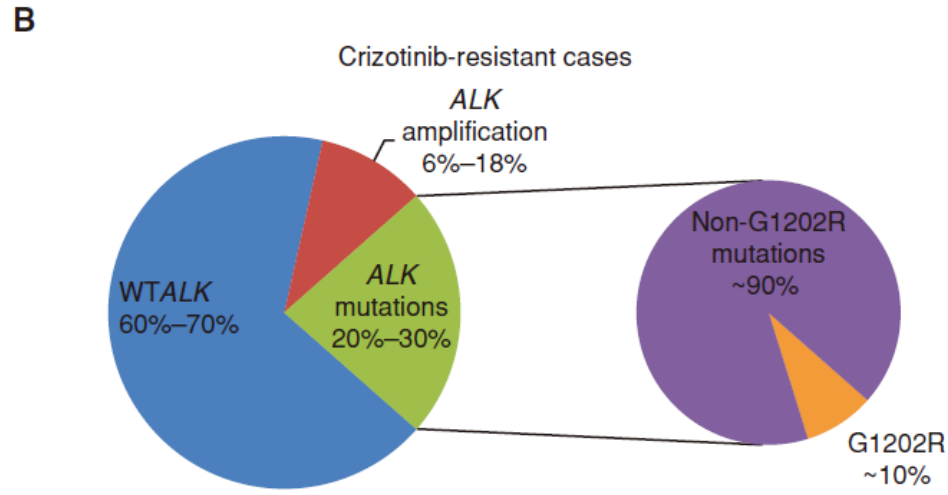
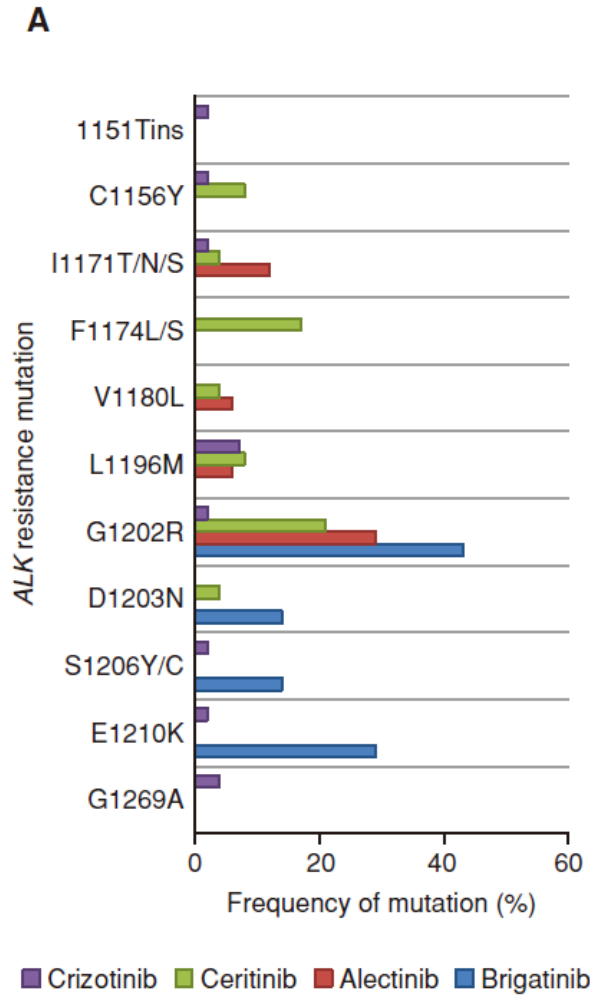
- **Prospective collection only for some samples**
 - Not sufficient samples **at diagnosis (before systemic treatment)**
 - Prospective validation for initial screening is warranted
- **Total RNA amount : Plasma/Platelets << FFPE tissue**
 - Variations in tumor characteristics (i.e., shedding vs non-shedding) or patient factors
 - Needs for **more sensitive platforms** for liquid biopsy (e.g., ddPCR, NGS)
- **Quantification of the result of RT-PCR**
- **Genotyping for EML4-ALK fusion variants**
 - High rates of invalid results (plasma, 46.8%; platelets, 53.4%)

Future Perspectives

: 1L NG ALK-TKIs & Resistance mutation

- Selection of 1L ALK inhibitor : Crizotinib or Alectinib or Ceretinib
 - Overall / CNS efficacy
 - ALK variants
 - **Acquired resistance mutation : Rebiopsy (Tissue, Liquid)**
- Subsequent treatment
 - Crizotinib, Alectinib, Ceretinib, Brigatinib, Lorlatinib, Entrectinib, Ensartinib

Acquired Resistance to ALK inhibitors

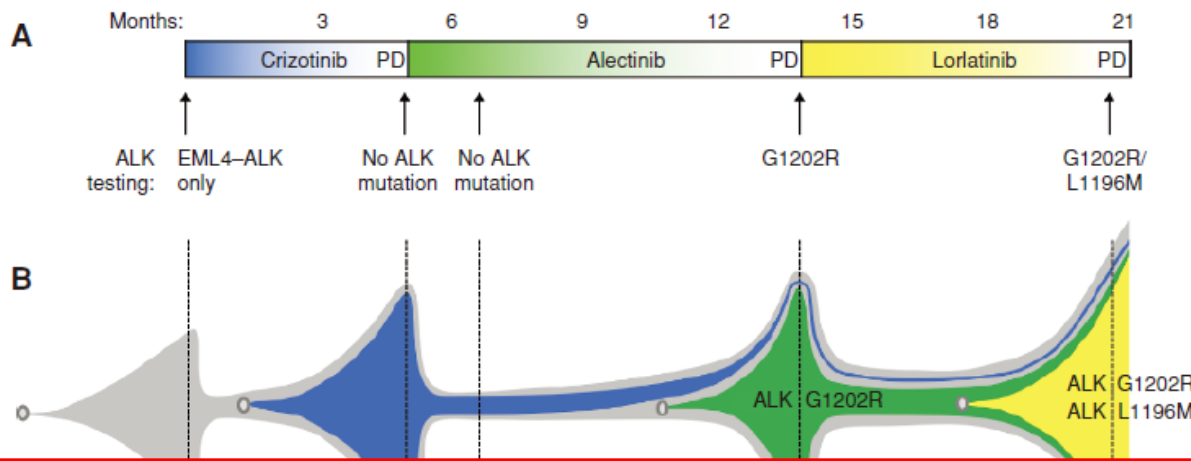


Sequential ALK Inhibitors Can Select for Lorlatinib-Resistant Compound ALK Mutations in ALK-Positive Lung Cancer

Satoshi Yoda^{1,2}, Jessica J. Lin^{1,2}, Michael S. Lawrence^{1,2,3}, Benjamin J. Burke⁴, Luc Friboulet⁵, Adam Langenbacher^{1,2,3}, Leila Dardaei^{1,2}, Kylie Prutisto-Chang¹, Ibiayi Dagogo-Jack^{1,2}, Sergei Timofeevski⁴, Harper Hubbeling^{1,2}, Justin F. Gainor^{1,2}, Lorin A. Ferris^{1,2}, Amanda K. Riley¹, Krystina E. Kattermann¹, Daria Timonina¹, Rebecca S. Heist^{1,2}, A. John Iafrate⁶, Cyril H. Benes^{1,2}, Jochen K. Lennerz⁶, Mari Mino-Kenudson⁶, Jeffrey A. Engelman⁷, Ted W. Johnson⁴, Aaron N. Hata^{1,2}, and Alice T. Shaw^{1,2}

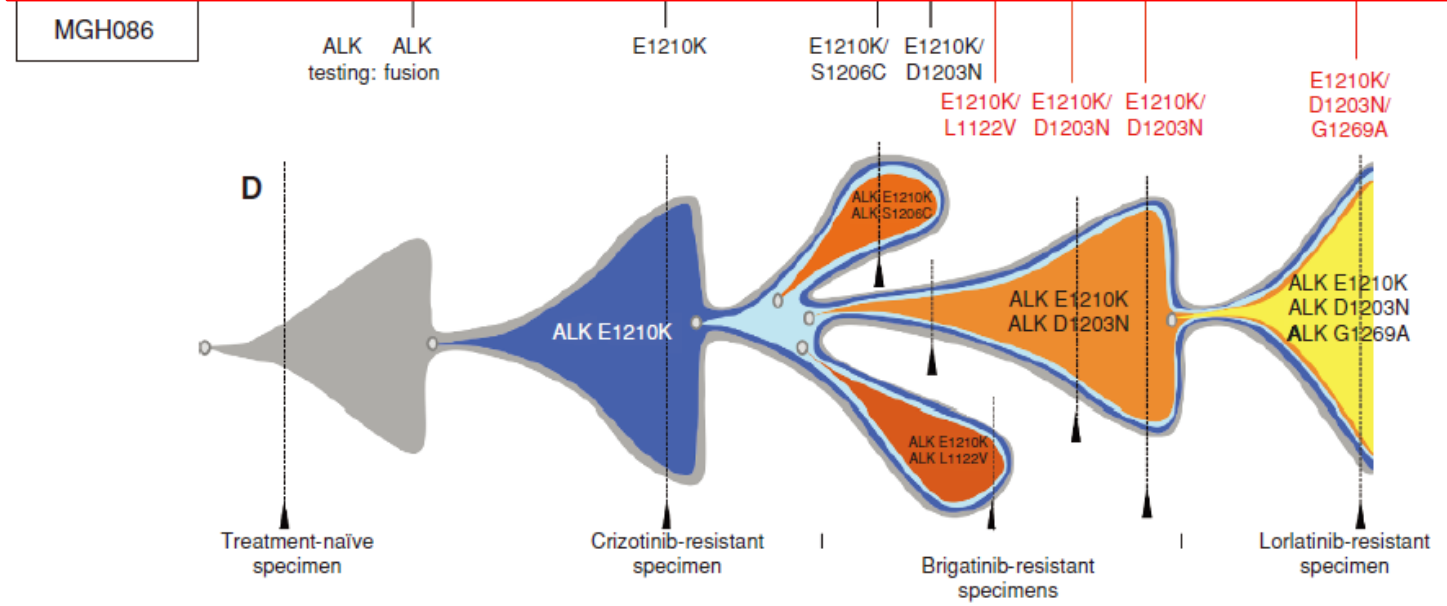
Table 1. ALK mutations in pre- and post-lorlatinib biopsies

Patient ID	Resistance ^a	Pre-lorlatinib ^b	Post-lorlatinib
MGH947	Primary	—	No ALK mutation
MGH048	Primary	—	No ALK mutation
MGH962	Primary	—	No ALK mutation
MGH952	Primary	No ALK mutation	No ALK mutation
MGH098	Primary	No ALK mutation	No ALK mutation
MGH964	Primary	No ALK mutation	No ALK mutation
MGH9107	Primary	No ALK mutation	No ALK mutation
MGH987	Primary	—	ALK I1171N + L1198F ^c
MGH990	Acquired	—	ALK I1171N + D1203N
MGH9041	Acquired	—	ALK G1202R + G1269A
MGH062	Acquired	ALK C1156Y	ALK C1156Y + L1198F ^d
MGH953	Acquired	ALK G1202R	ALK G1202R + L1196M ^e
MGH087	Acquired	ALK G1202R	ALK G1202R + L1204V + G1269A ^e
MGH086	Acquired	ALK E1210K + D1203N	ALK E1210K + D1203N + G1269A ^e
MGH065	Acquired	ALK L1196M	ALK G1269A
MGH9092	Acquired	ALK I1171N	No ALK mutation
MGH040	Acquired	ALK G1202R	No ALK mutation
MGH9094	Acquired	—	No ALK mutation
MGH9106	Acquired	—	No ALK mutation
MGH9108	Acquired	—	No ALK mutation



ABSTRACT The cornerstone of treatment for advanced ALK-positive lung cancer is sequential therapy with increasingly potent and selective ALK inhibitors. The third-generation ALK inhibitor lorlatinib has demonstrated clinical activity in patients who failed previous ALK inhibitors. To define the spectrum of ALK mutations that confer lorlatinib resistance, we performed accelerated mutagenesis screening of Ba/F3 cells expressing EML4-ALK. Under comparable conditions, N-ethyl-N-nitrosourea (ENU) mutagenesis generated numerous crizotinib-resistant but no lorlatinib-resistant clones harboring single ALK mutations. In similar screens with EML4-ALK containing single ALK resistance mutations, numerous lorlatinib-resistant clones emerged harboring compound ALK mutations. To determine the clinical relevance of these mutations, we analyzed repeat biopsies from lorlatinib-resistant patients. Seven of 20 samples (35%) harbored compound ALK mutations, including two identified in the ENU screen. Whole-exome sequencing in three cases confirmed the stepwise accumulation of ALK mutations during sequential treatment. These results suggest that sequential ALK inhibitors can foster the emergence of compound ALK mutations, identification of which is critical to informing drug design and developing effective therapeutic strategies.

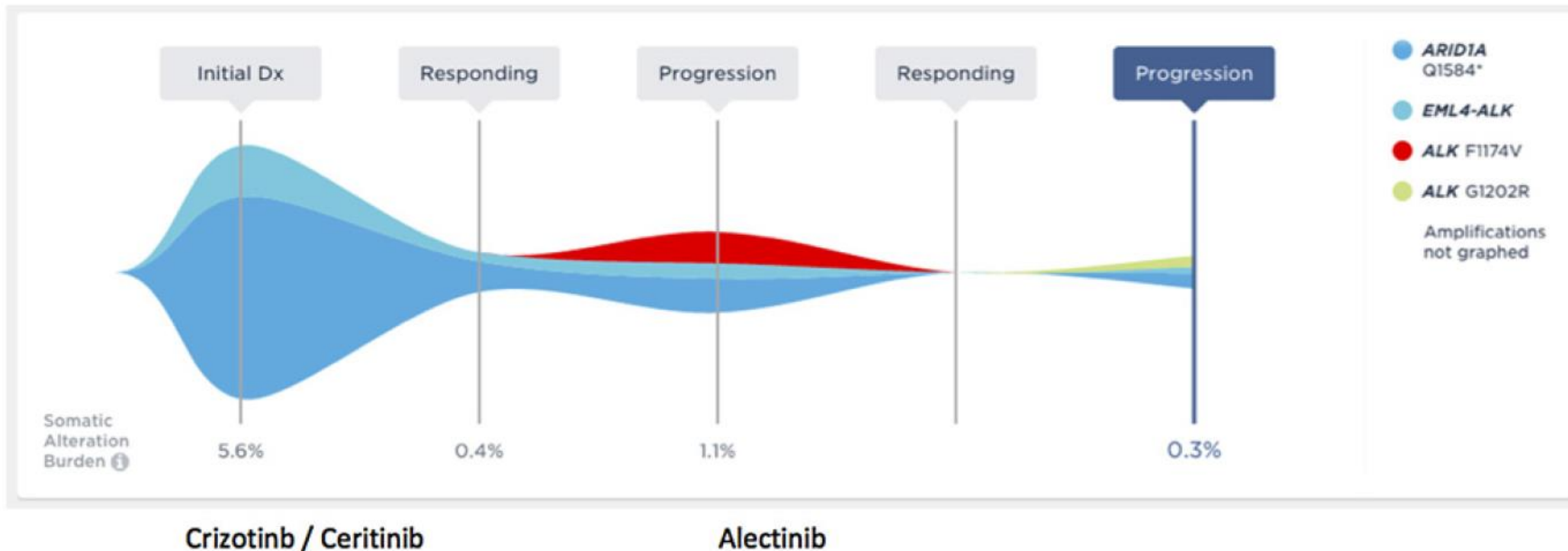
SIGNIFICANCE: Treatment with sequential first-, second-, and third-generation ALK inhibitors can select for compound ALK mutations that confer high-level resistance to ALK-targeted therapies. A more efficacious long-term strategy may be up-front treatment with a third-generation ALK inhibitor to prevent the emergence of on-target resistance. *Cancer Discov; 8(6); 714-29. ©2018 AACR.*



Clinical Utility of Cell-Free DNA for the Detection of *ALK* Fusions and Genomic Mechanisms of *ALK* Inhibitor Resistance in Non-Small Cell Lung Cancer

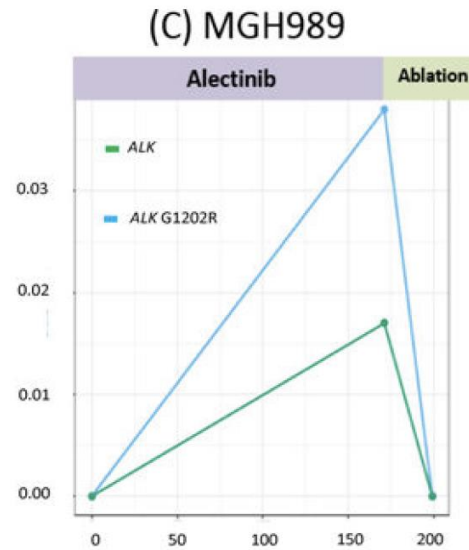
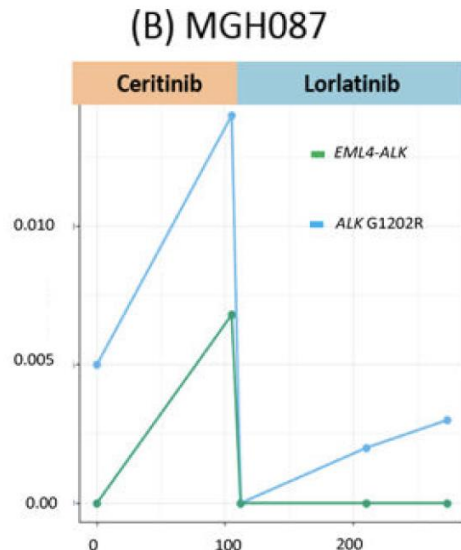
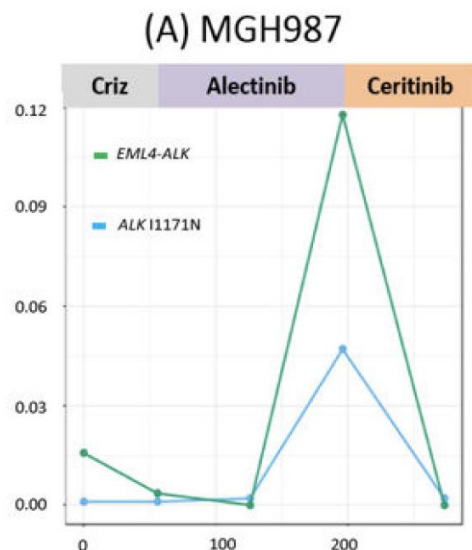


Caroline E. McCoach¹, Collin M. Blakely¹, Kimberly C. Banks², Benjamin Levy³, Ben M. Chue⁴, Victoria M. Raymond², Anh T. Le⁵, Christine E. Lee², Joseph Diaz², Saiama N. Waqar⁶, William T. Purcell⁵, Dara L. Aisner⁵, Kurtis D. Davies⁵, Richard B. Lanman², Alice T. Shaw⁷, and Robert C. Doebele⁵

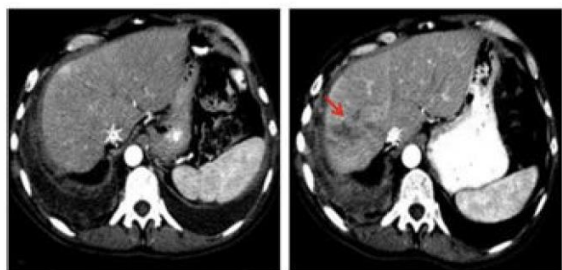




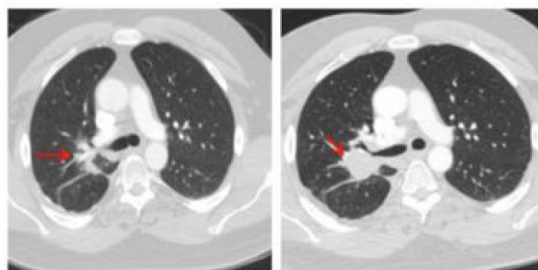
Tracking the evolution of resistance to ALKi



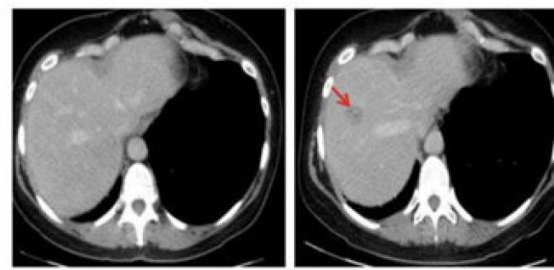
Quantitative assessment of structural variants may be clinically useful and complementary to radiographic assessment in some patients



Appearance of new liver lesions on alectinib



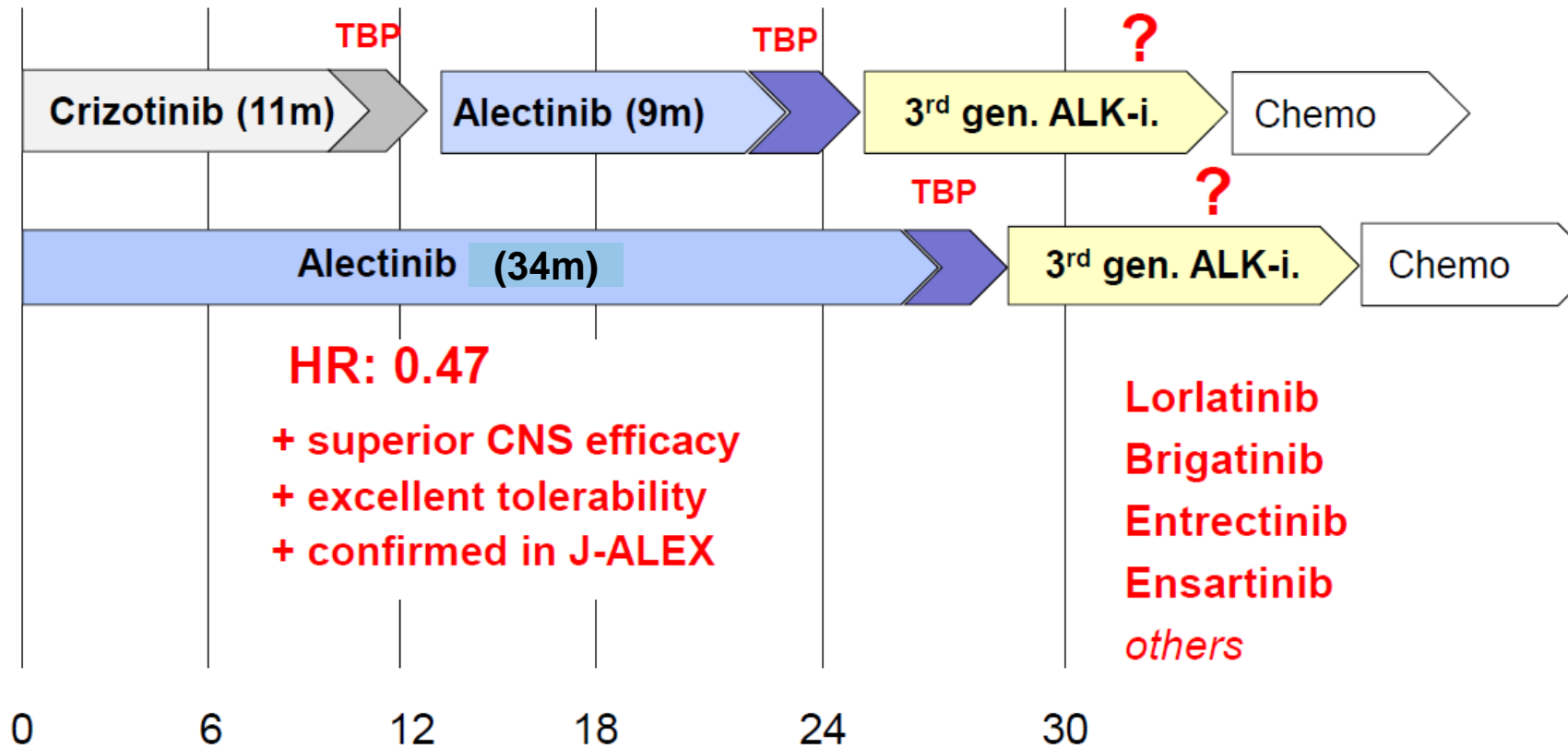
Progression of lung mass on ceritinib



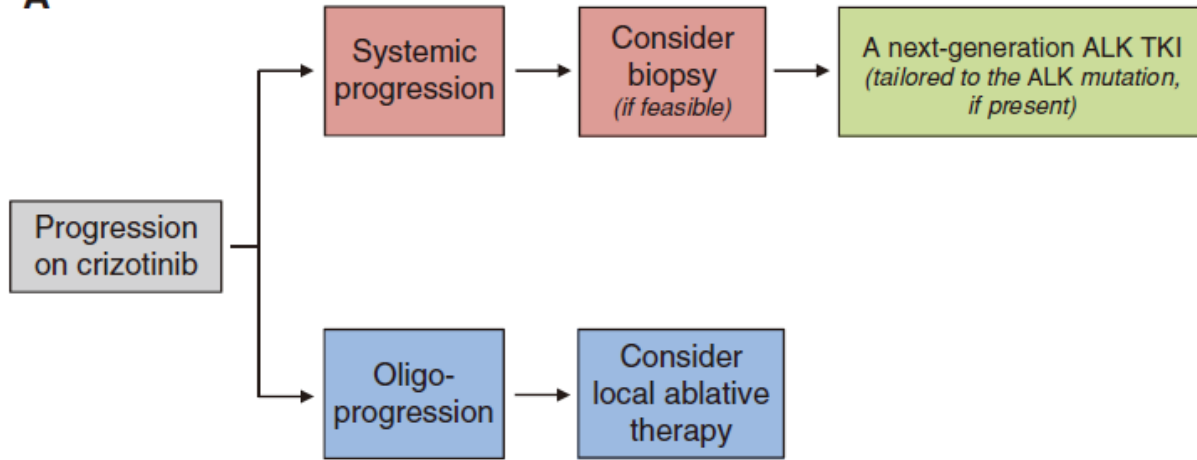
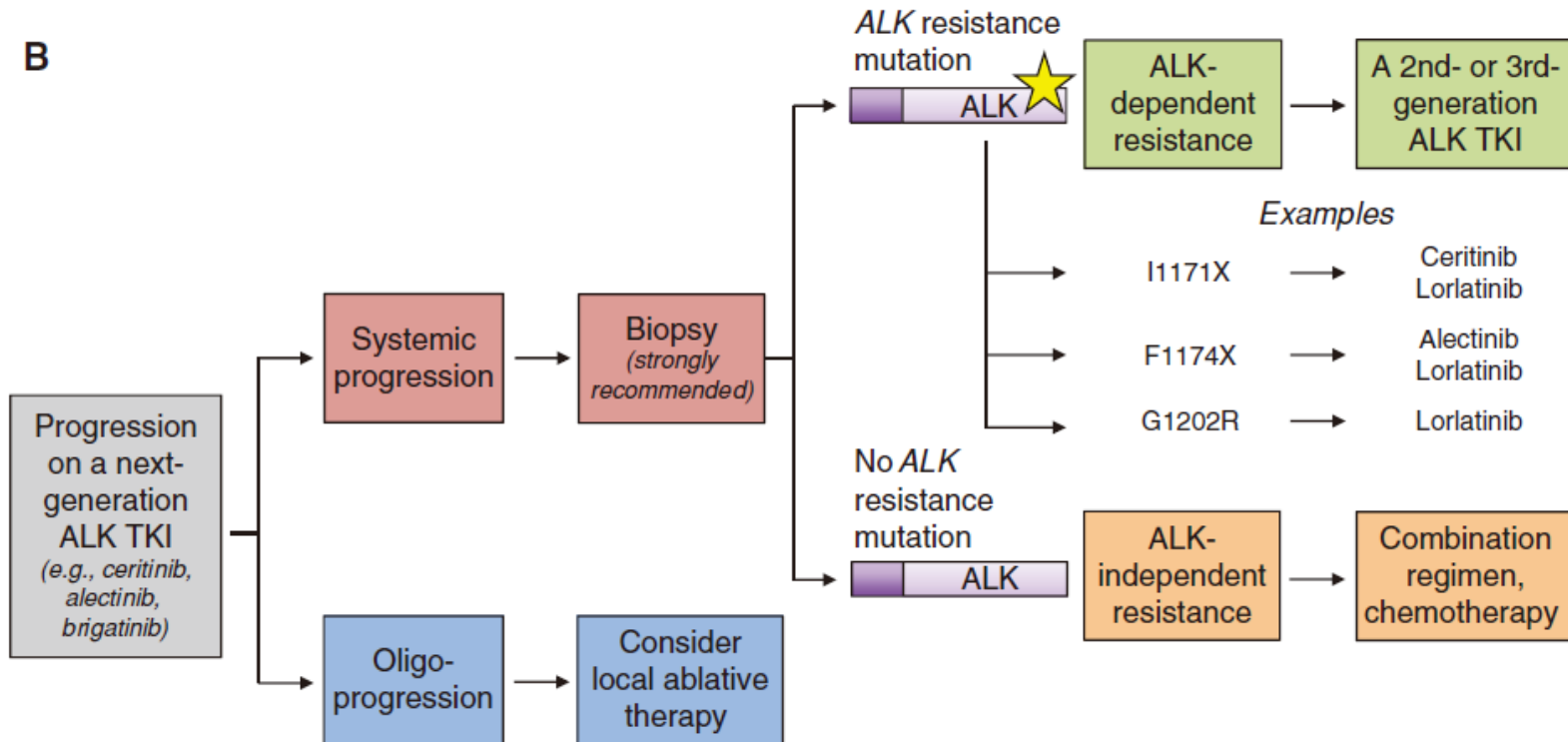
Liver oligoprogression on alectinib

Sequential therapy with ALK-inhibitors: OS

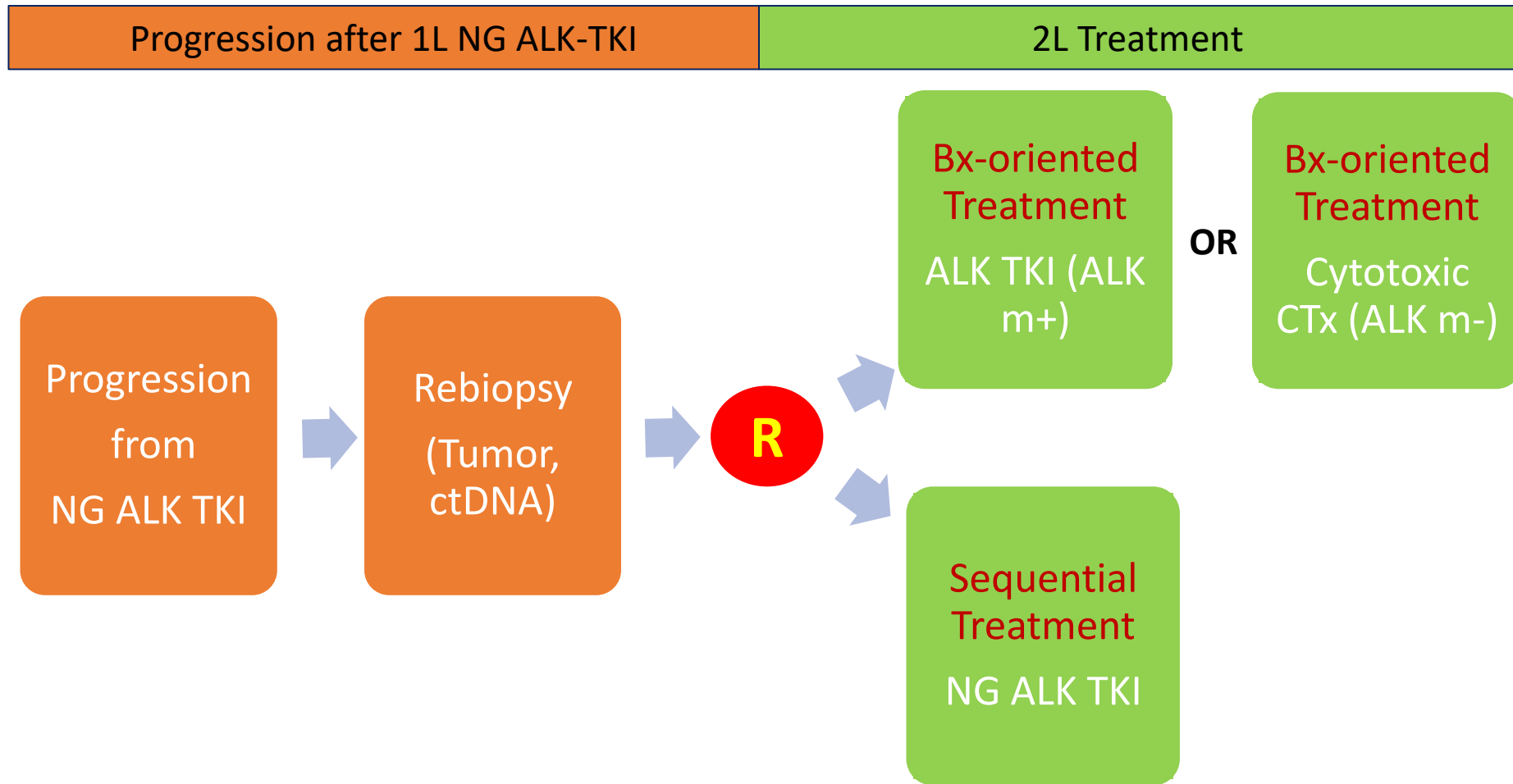
What is the role of other next. gen. ALK inhibitors ?



TBP : treatment beyond progression

A**B**

Future Perspectives



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THANK YOU FOR YOUR ATTENTION !!