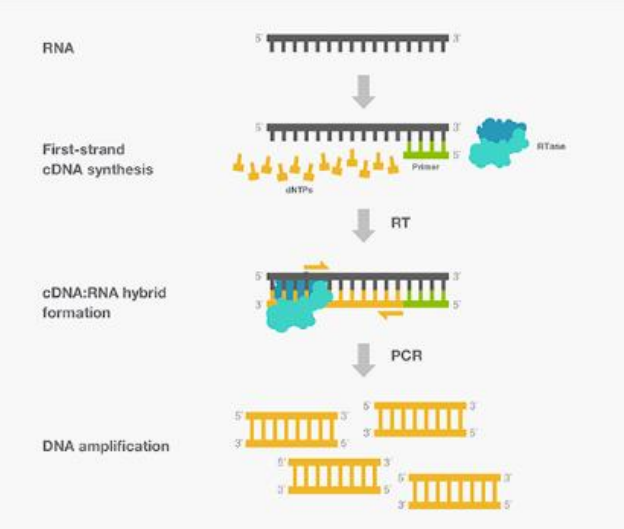
# Blood-Based Response Monitoring and Detection of Acquired Resistance to ALK Inhibitors in ALK-Positive NSCLC

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**2018 KATRD Winter Workshop**

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# Contents

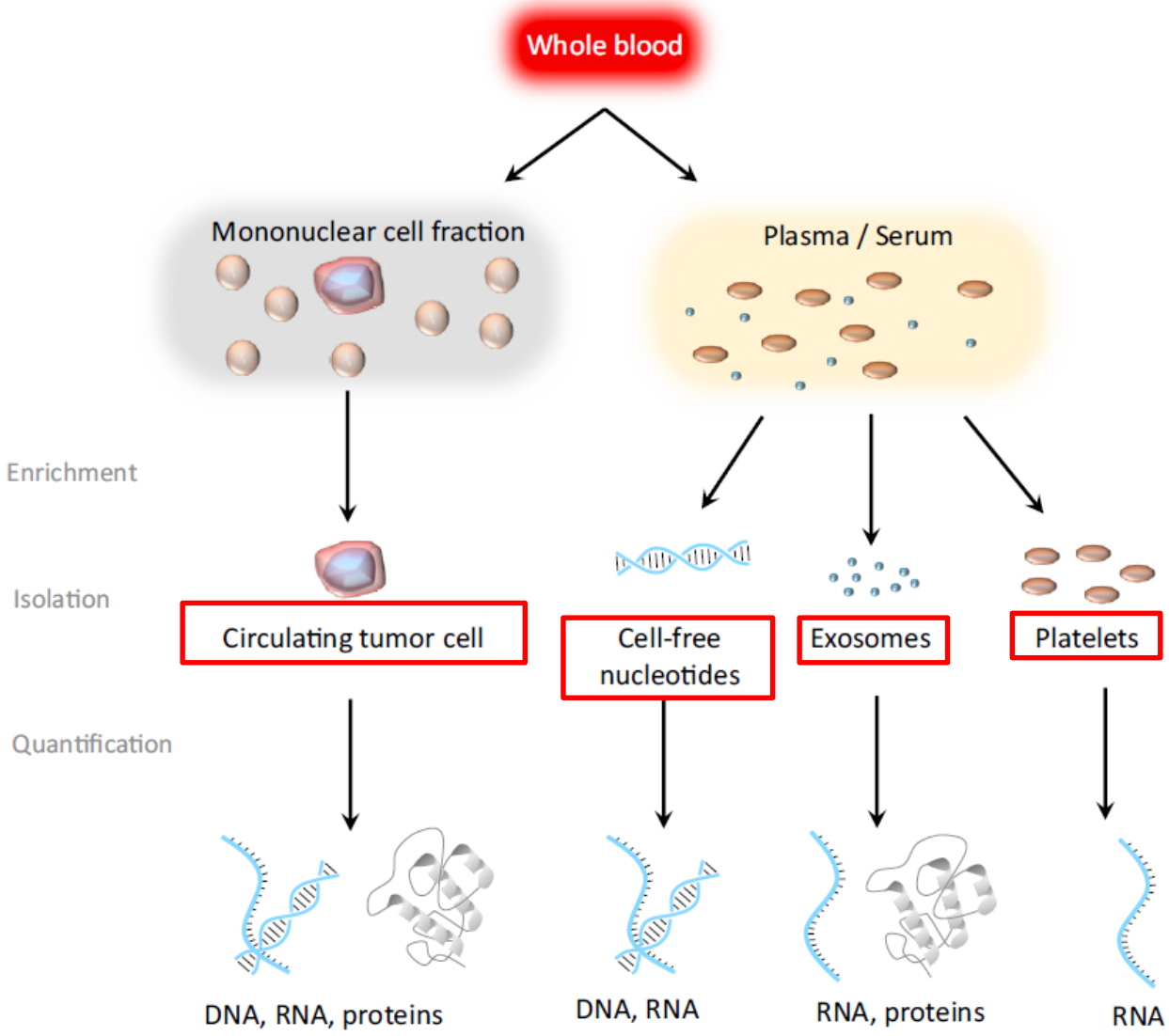
- Scientific Rationale
- Hypothesis (Objectives), Endpoints
- Study Design
- Study Subjects
- Methods, Assessment
- Future Directions

# Liquid Biopsy in Lung Cancer

- Role of Liquid Biopsy in Advanced Lung Cancer
  - Initial detection of actionable oncogenic drivers
  - Identification of resistance mutations in relapsed patients on targeted therapies
  - Monitoring of response to therapy
  - Prediction of clinical outcome

Cancers 2017;9:106

# Sources of Liquid Biopsy (Blood)




Cancer Cell 2015;28(5):552-4

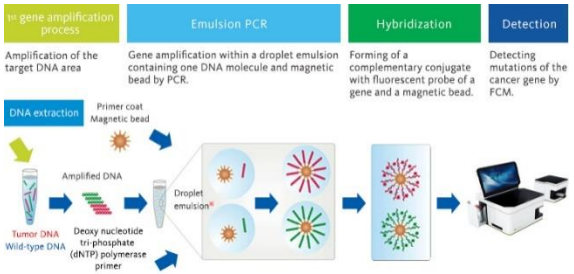
# Detection Methods of Liquid Biopsy

PCR-based Methods

ddPCR


Cobas v2


PANA Mutyper



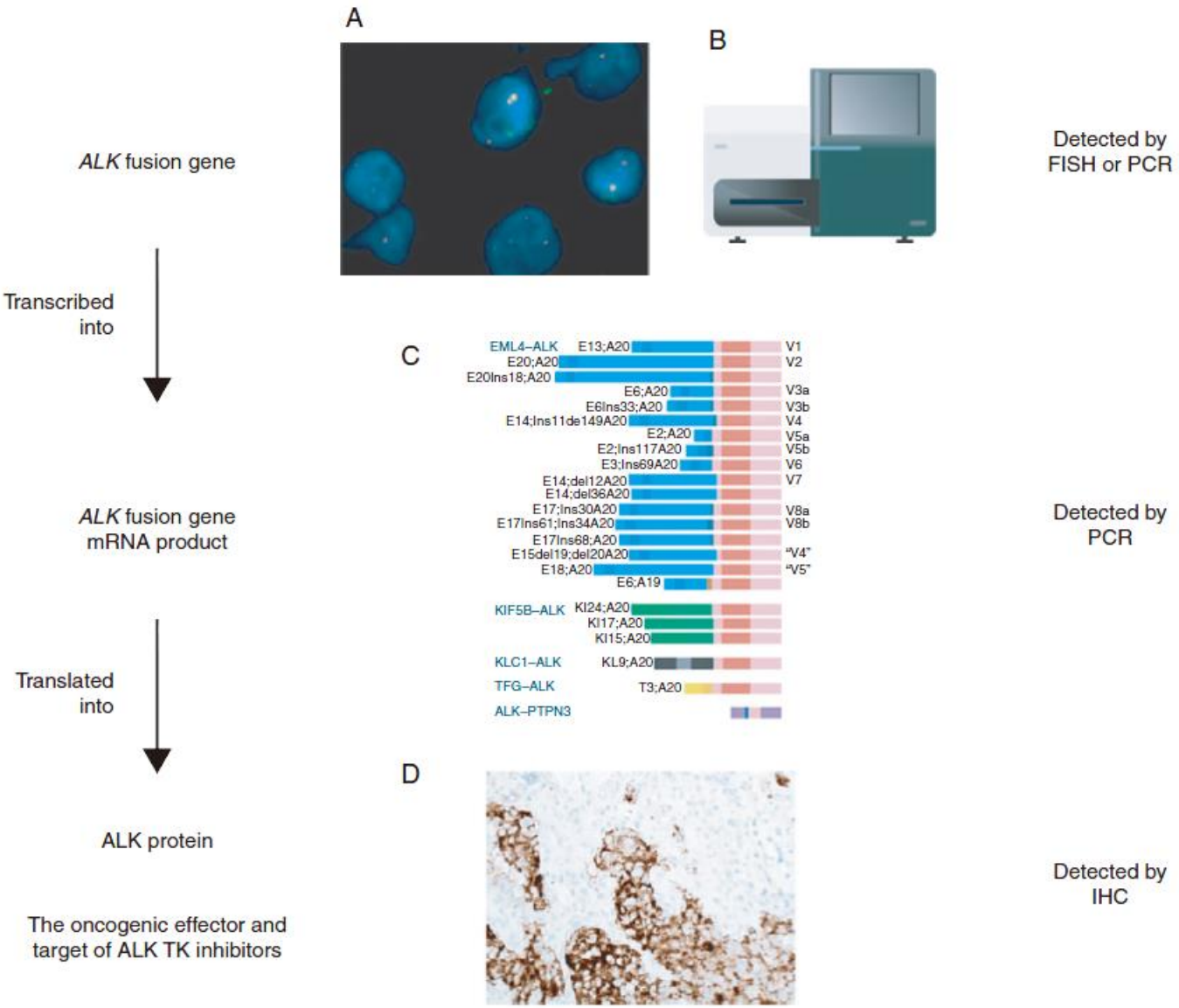

BEAMing

Next generation Sequencing

Ion Proton
(Thermo Fisher)

Next-Seq
(Illumina)

# Diagnosis of ALK+ NSCLC



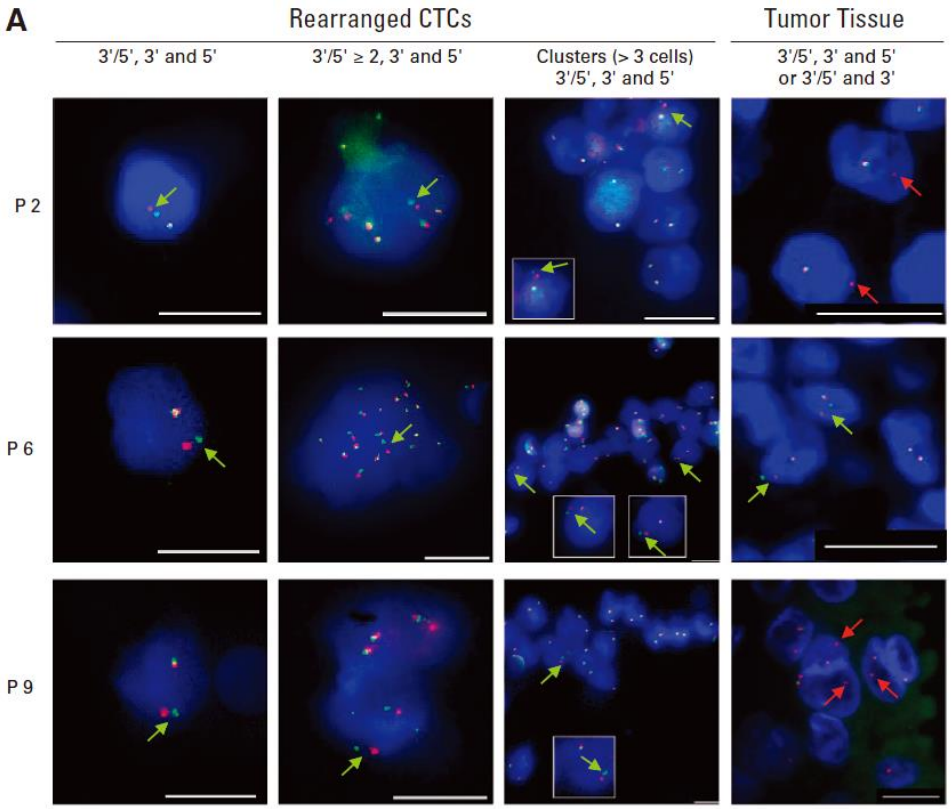

Ann Oncol 2016;27(Suppl 3):iii16-iii24

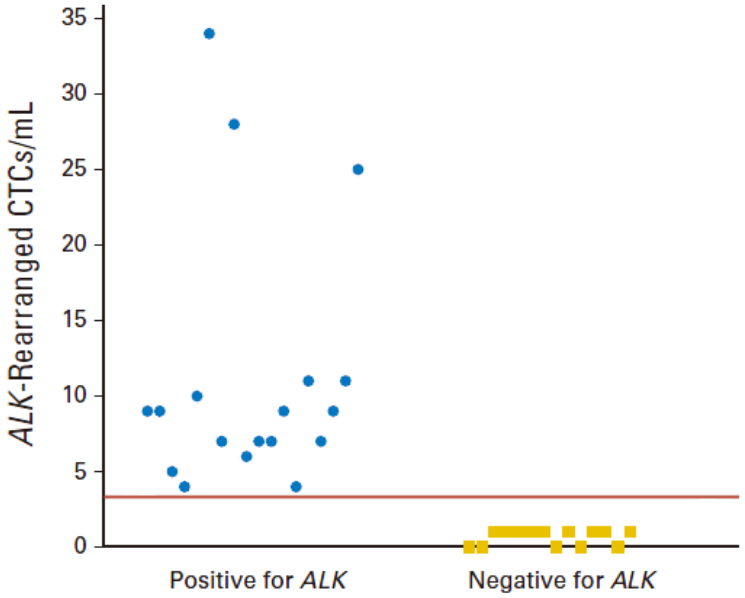
# Detection of ALK by Liquid Biopsy – CTC

FISH, IHC, PCR, NGS

## Detection of Circulating Tumor Cells Harboring a Unique *ALK* Rearrangement in *ALK*-Positive Non–Small-Cell Lung Cancer

*Emma Pailler, Julien Adam, Amélie Barthélémy, Marianne Oulhen, Nathalie Auger, Alexander Valent, Isabelle Borget, David Planchard, Melissa Taylor, Fabrice André, Jean Charles Soria, Philippe Vielh, Benjamin Besse, and Françoise Farace*




| *ALK*-Rearranged CTCs | *ALK* Status in Tumor Samples | | Total | |
|---|---|---|---|---|
| | Patients Positive for *ALK* | Patients Negative for *ALK* | | |
| < 4 | 0 | 14 | 14 | NPV = 100% |
| ≥ 4 | 18 | 0 | 18 | PPV = 100% |
| Total | 18 | 14 | 32 | |
| | Sensitivity = 100% | Specificity = 100% | | |

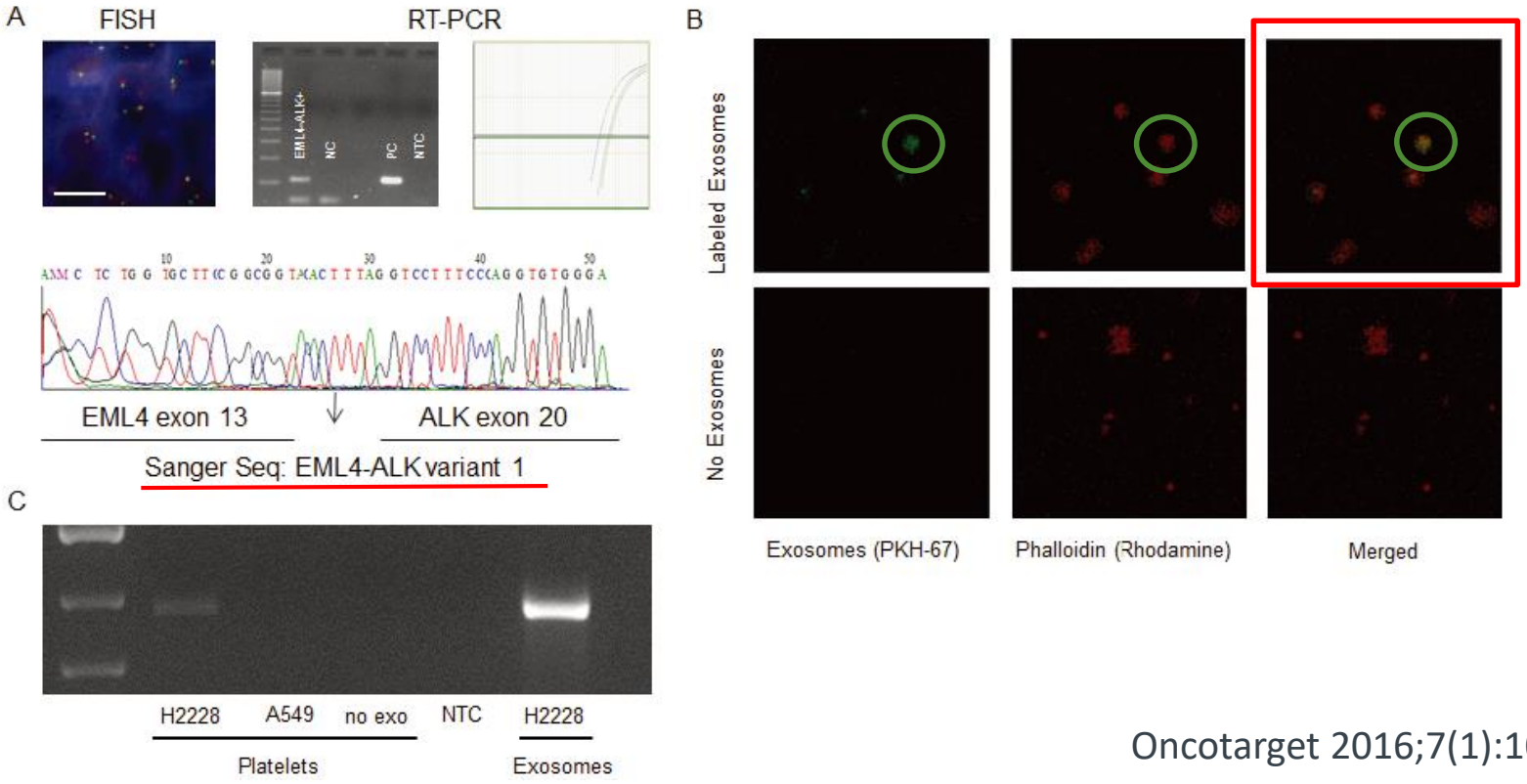
J Clin Oncol 2013;31(18):2273-81

# Detection of ALK by Liquid Biopsy – Plasma, Platelet

PCR, NGS

## 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 Berenguer[1], Ana Gimenez-Capitan[5], Pepijn Schellen[1,3], Cristina Teixido[5], Jihane Tannous[6], Justine L. Kuiper[7], Esther Drees[1], Magda Grabowska[1], Marte van Keulen[6], Danielle A. M. Heideman[8], Erik Thunnissen[8], Anne-Marie C. Dingemans[9], Santiago Viteri[4], Bakhos A. Tannous[6], Ana Drozdowskyj[10], Rafael Rosell[4,5,11,12,**], Egbert F. Smit[7,**] and Thomas Wurdinger[1,3,6,**]



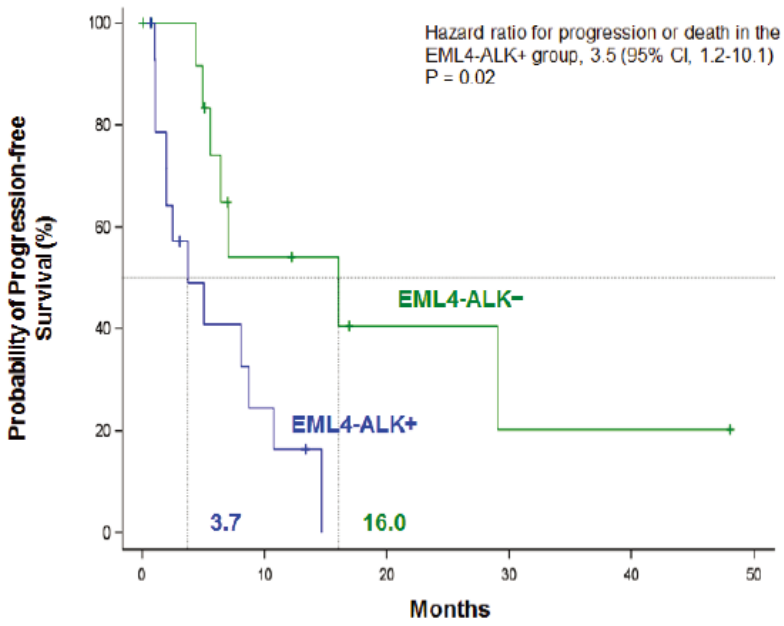

Oncotarget 2016;7(1):1066-75

# Detection of ALK by Liquid Biopsy – Plasma, Platelet

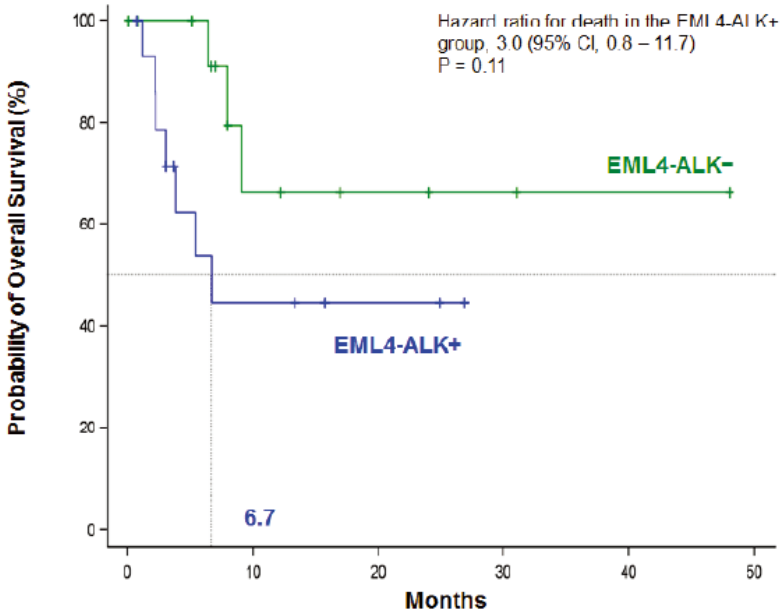
PCR, NGS

## ❖ Prognostic Factor

- Blood sampling : at baseline and/or during treatment
- Platelet : EML4-ALK+ (+/+ or +/0 or 0/+), EML4-ALK- (+/- or -/0 or 0/-)




PFS



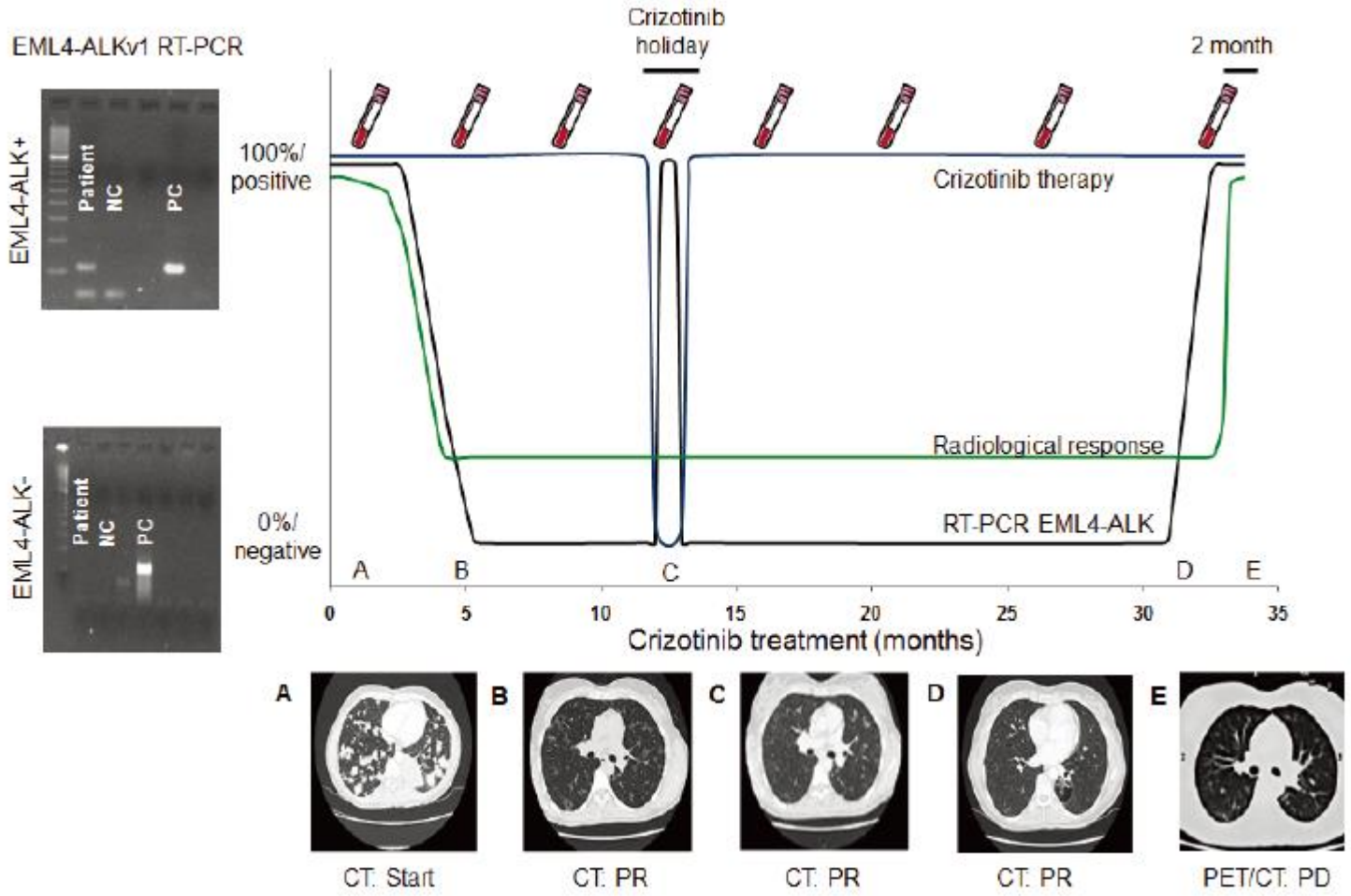

OS

Oncotarget 2016;7(1):1066-75

# Detection of ALK by Liquid Biopsy – Plasma, Platelet

PCR, NGS

- Longitudinal Monitoring of Crizotinib Response



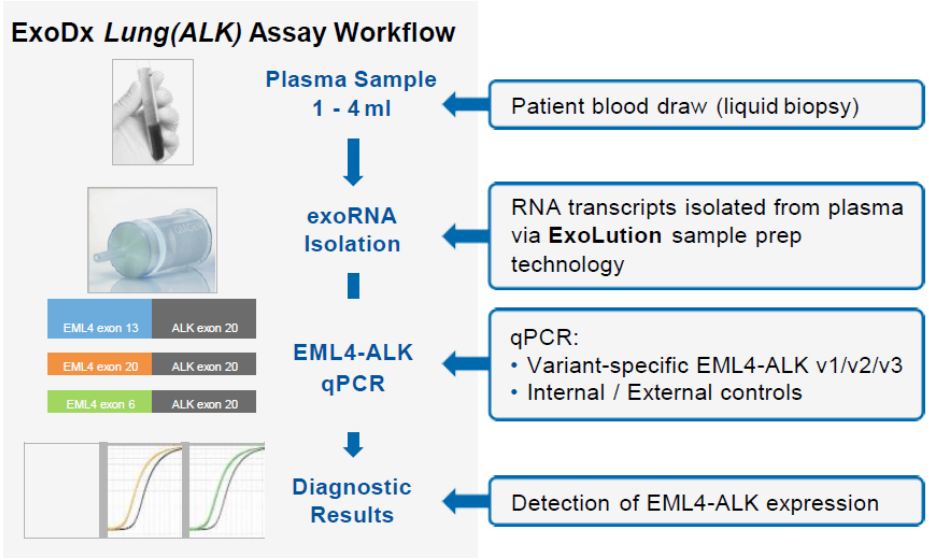

Oncotarget 2016;7(1):1066-75

# Detection of ALK by Liquid Biopsy – Exosome

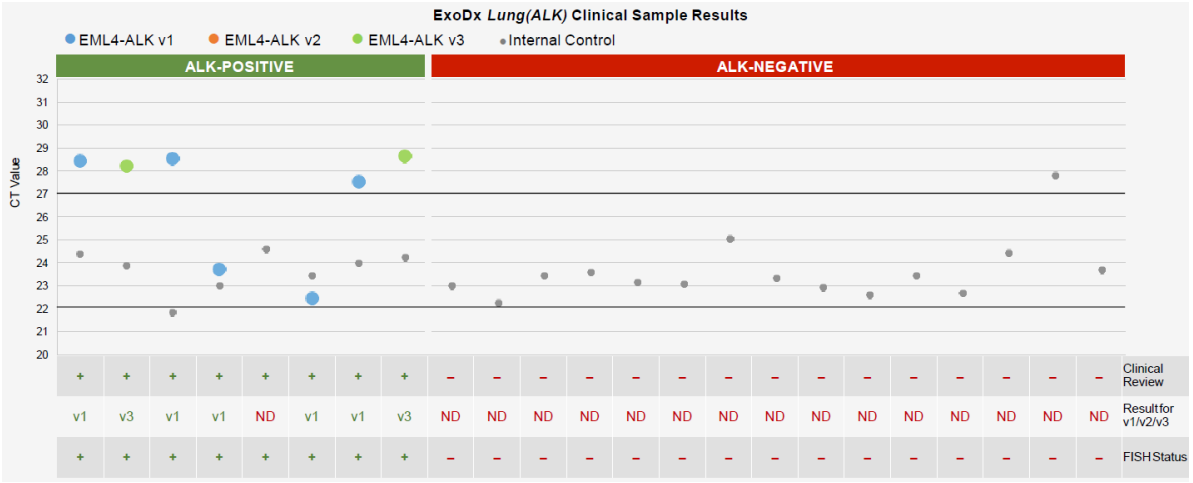
PCR, NGS

## Exosomal RNA-based liquid biopsy detection of EML4-ALK in plasma from NSCLC patients

Brinkmann K [1], Emenegger J [1], Hurley J [2], Castellanos-Rizaldos E [2], Enderle D [1], Koestler T [1], Spiel A [1], Mueller R [1], Baughman D [2] Brock G [2], O'Neill V [2], Skog J [2]

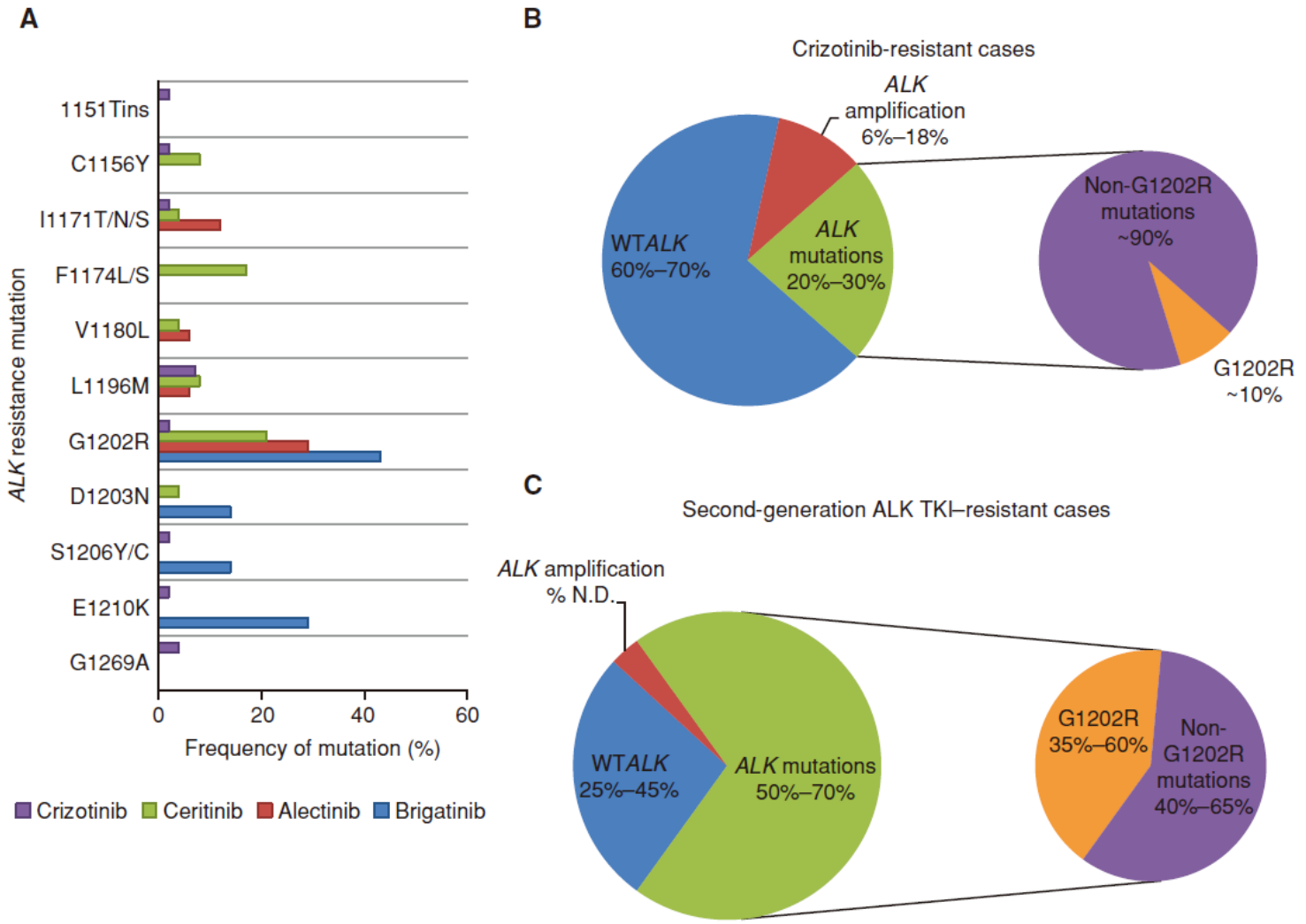



| | | Samples defined by Clinical Review | |
|---|---|---|---|
| | | Pos | Neg |
| ExoDx Lung (ALK) | Pos | 7 | 0 |
| | Neg | 1 | 15 |
| | | Sensitivity [%] | Specificity [%] |
| | | 88% | 100% |



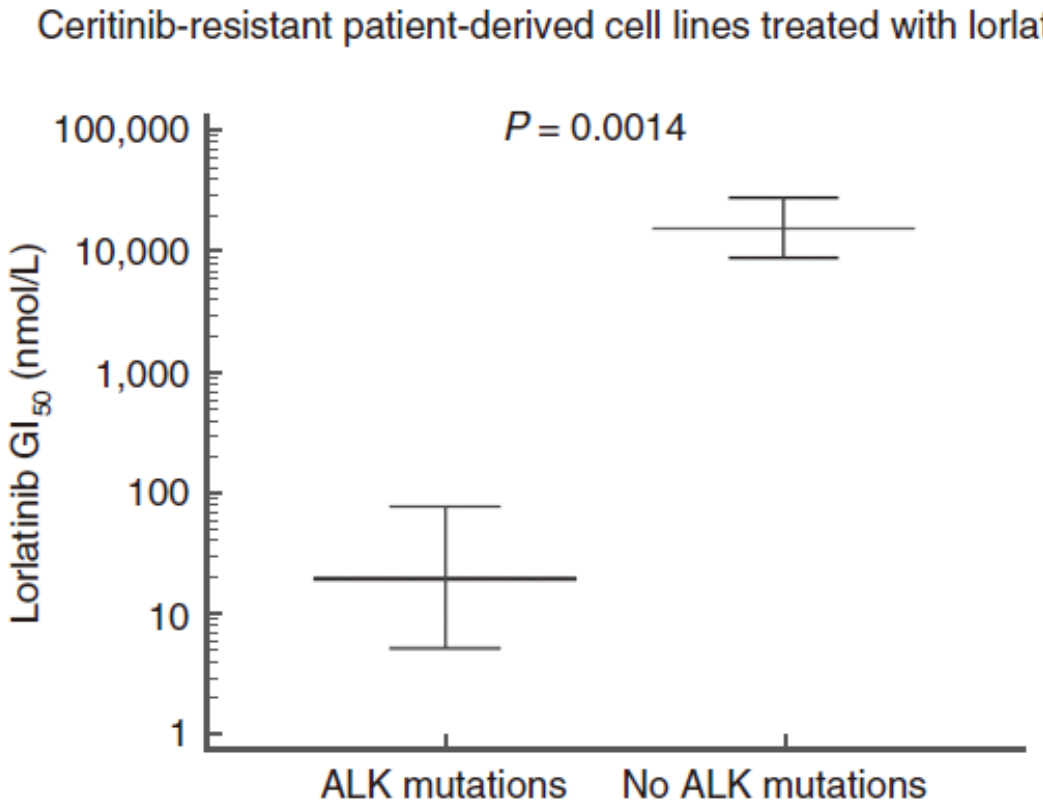

NCCN 2016

# Mechanism of Acquired Resistance to ALK Inhibitors




Cancer Discovery 2017

# Resistant Tumors without Secondary ALK Mutation May Be ALK-independent




Cancer Discovery 2016

# Mechanism of Acquired Resistance to ALK Inhibitors

| Mutation status | Cellular ALK phosphorylation mean $IC_{50}$ (nmol/L) | | | | |
|---|---|---|---|---|---|
| | Crizotinib | Ceritinib | Alectinib | Brigatinib | Lorlatinib |
| Parental Ba/F3 | 763.9 | 885.7 | 890.1 | 2774.0 | 11293.8 |
| *EML4–ALK* V1 | 38.6 | 4.9 | 11.4 | 10.7 | 2.3 |
| *EML4–ALK* C1156Y | 61.9 | 5.3 | 11.6 | 4.5 | 4.6 |
| *EML4–ALK* I1171N | 130.1 | 8.2 | 397.7 | 26.1 | 49.0 |
| *EML4–ALK* I1171S | 94.1 | 3.8 | 177.0 | 17.8 | 30.4 |
| *EML4–ALK* I1171T | 51.4 | 1.7 | 33.6[a] | 6.1 | 11.5 |
| *EML4–ALK* F1174C | 115.0 | 38.0[a] | 27.0 | 18.0 | 8.0 |
| *EML4–ALK* L1196M | 339.0 | 9.3 | 117.6 | 26.5 | 34.0 |
| *EML4–ALK* L1198F | 0.4 | 196.2 | 42.3 | 13.9 | 14.8 |
| *EML4–ALK* G1202R | 381.6 | 124.4 | 706.6 | 129.5 | 49.9 |
| *EML4–ALK* G1202del | 58.4 | 50.1 | 58.8 | 95.8 | 5.2 |
| *EML4–ALK* D1203N | 116.3 | 35.3 | 27.9 | 34.6 | 11.1 |
| *EML4–ALK* E1210K | 42.8 | 5.8 | 31.6 | 24.0 | 1.7 |
| *EML4–ALK* G1269A | 117.0 | 0.4 | 25.0 | ND | 10.0 |
| *EML4–ALK* D1203N+F1174C | 338.8 | 237.8 | 75.1 | 123.4 | 69.8 |
| *EML4–ALK* D1203N+E1210K | 153.0 | 97.8 | 82.8 | 136.0 | 26.6 |

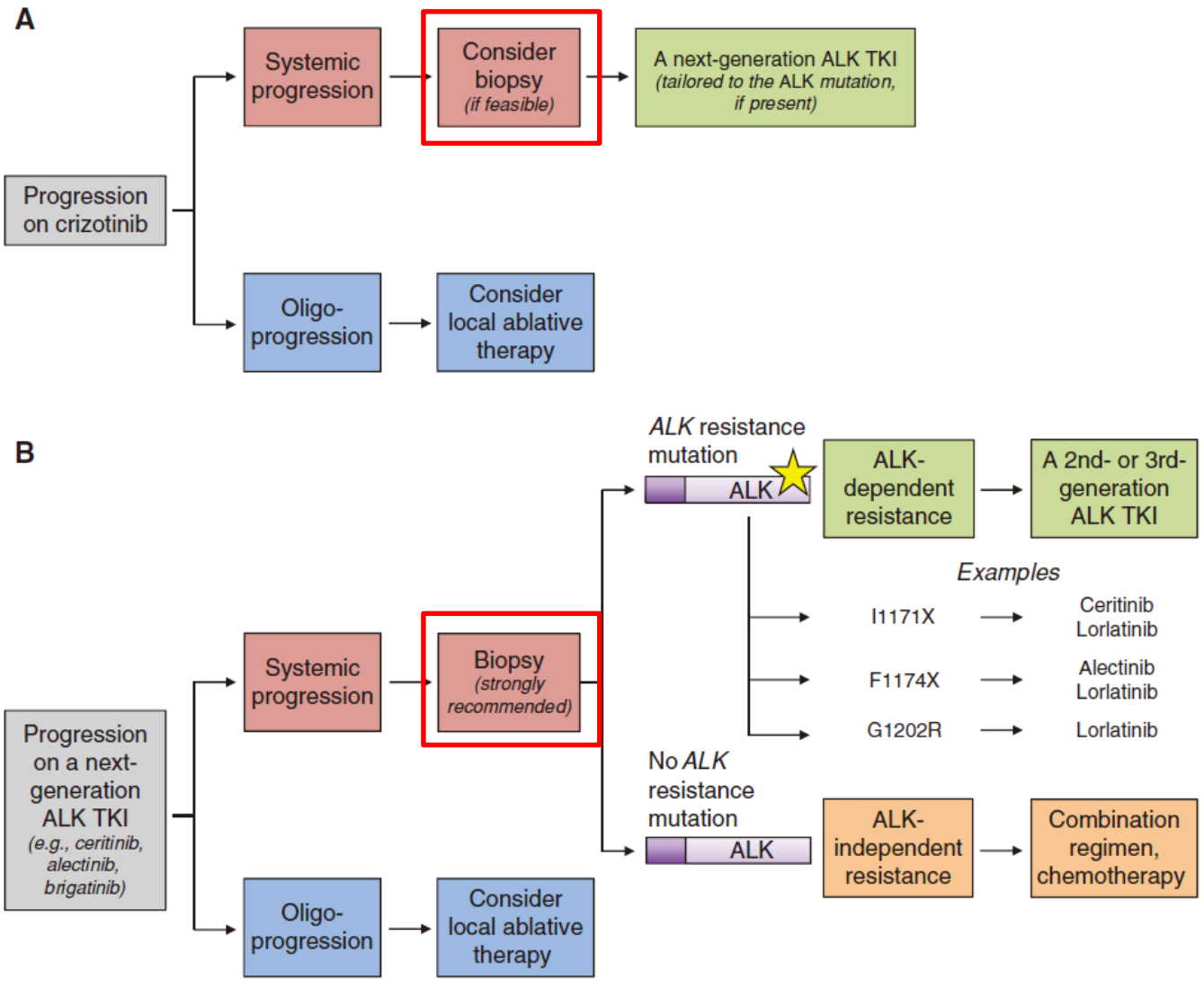
$IC_{50} \leq 50$ nmol/L

$IC_{50} > 50 < 200$ nmol/L

$IC_{50} \geq 200$ nmol/L

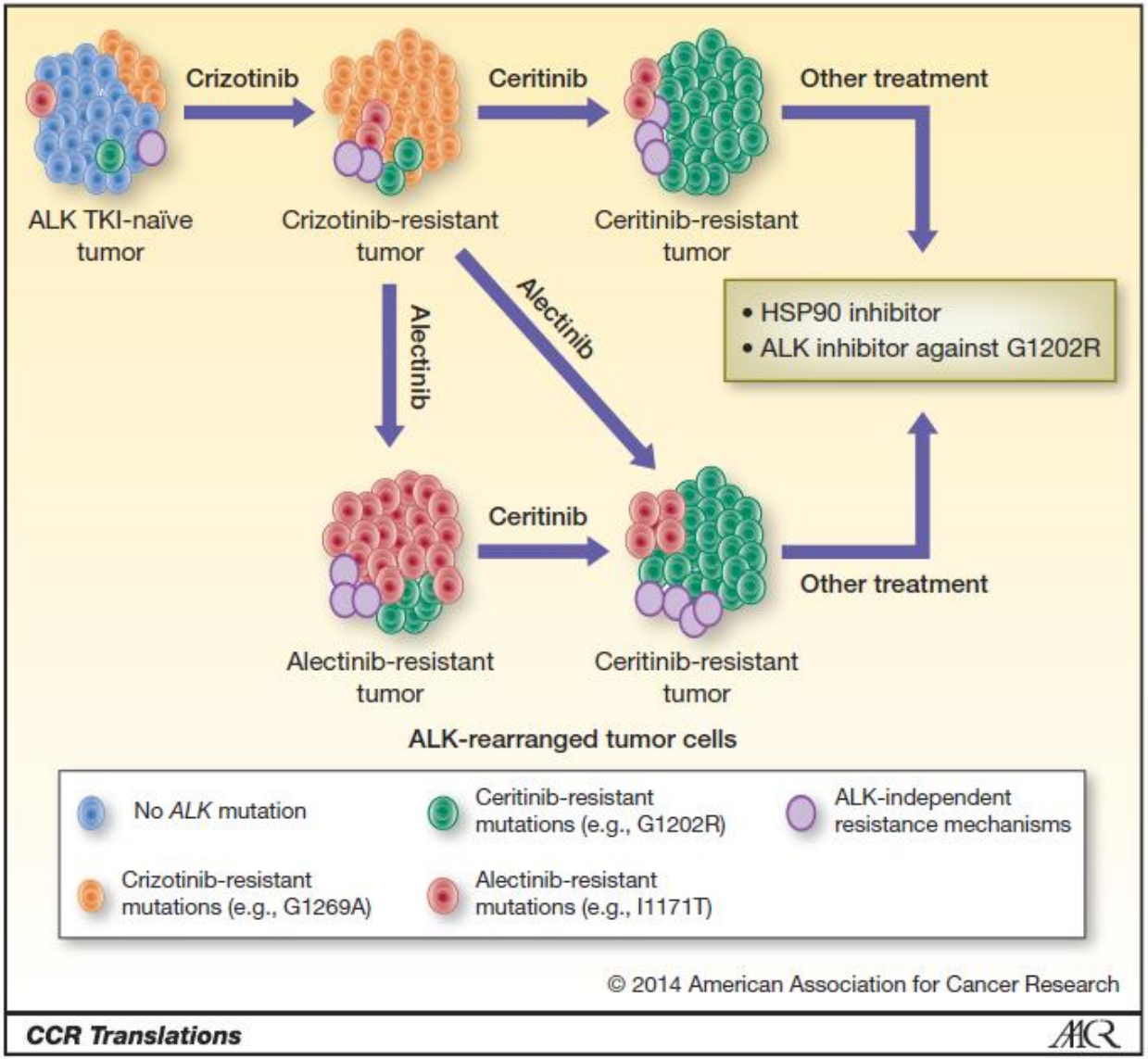
Cancer Discov 2016;6(10):1118-33

# Selecting Treatment after Progression on ALK Inhibitors




Cancer Discovery 2017

# Monitoring of Resistance during ALK TKIs Treatment




Plasma

PCR, NGS

Clin Cancer Res 2014;20(22): 1–3

# Hypothesis (Objectives), Endpoints

- Feasibility of **liquid biopsy** on **longitudinal monitoring** of response to ALK inhibitors by detection of ALK fusion transcript

  : **Source** – Plasma, Platelets, Exosome

  : **Technique** – RT-PCR

  : **Endpoints** – ORR/PFS/DoR between + or - conversion, persistent + or -

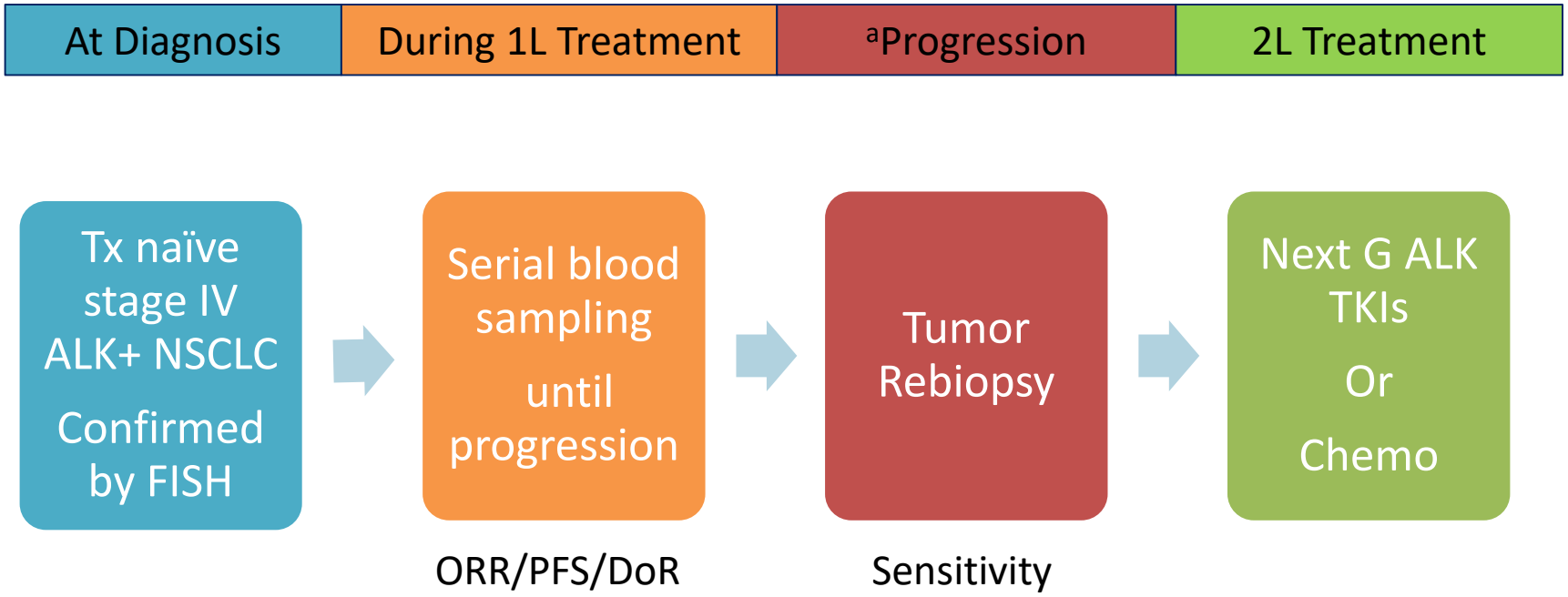
- Feasibility of **liquid biopsy** on detection of **acquired resistance mutation** after progression on ALK inhibitors

  : **Source** – Plasma

  : **Technique** – RT-PCR, NGS

  : **Endpoints** – Sensitivity in detection of ALK resistance mutation (validation with tissue)

# Study Design




[a]according to RECIST v1.1 or investigator`s assessment

# Study Subjects

- Treatment naïve, histologically confirmed, stage IV NSCLC
- Patients with ALK rearrangements confirmed by (tissue) FISH
- At least one measurable tumor lesion according to RECIST criteria v1.1[1]
- Platelets counts (≥100,000/$m^3$)

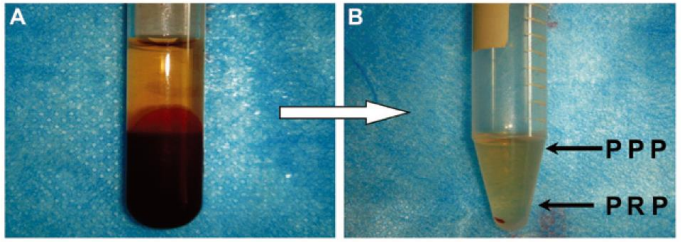
[1]Eur J Cancer 2009;45(2):228-47

# Methods – Screening

- Whole blood sampling (10ml or more) at diagnosis

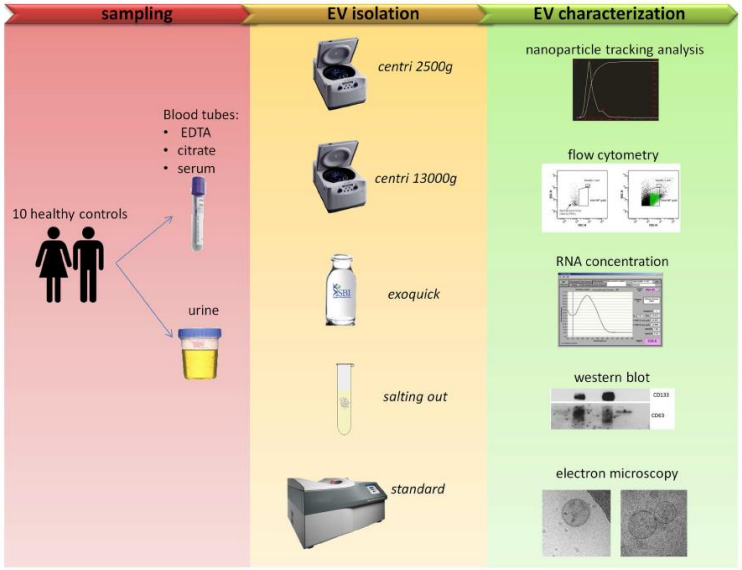
1) Whole blood : specific tubes for RNA preservation tube

2) Plasma/Platelets isolation, frozen at -80°C with RNAlater




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

3) Exosome




Front Immunol 2015;6:50

STRECK

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## CELL-FREE RNA BCT®

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A blood collection tube with a patented preservative, which stabilizes cell-free RNA in plasma and prevents the release of non-target background RNA from blood cells.

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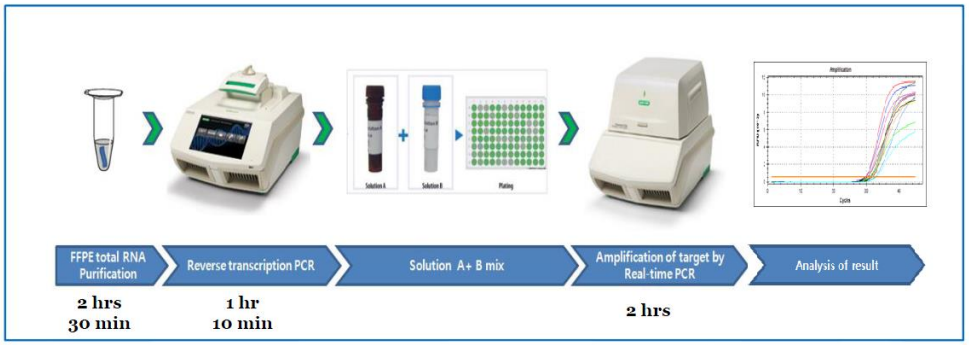
- Allows for conver

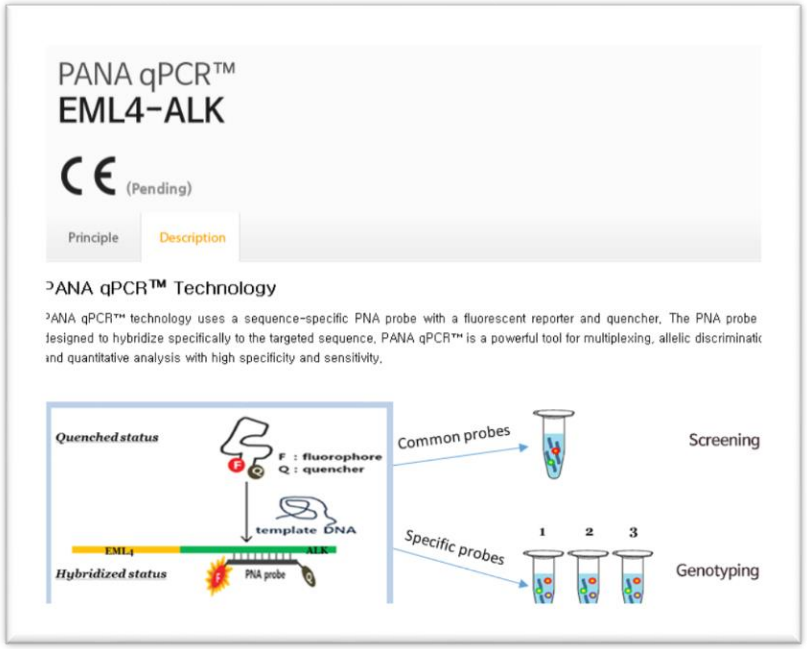
### Stable

- cfRNA is stable f
- Unfilled tubes hav

# Methods – Screening

- RNA extraction & RT-PCR assay (EML4-ALK Screening & Genotyping)




# Detection of ALK Rearrangements using RT-PCR

| No (%) | RT-PCR | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | FFPE | | | Plasma | | Platelets | | Liquid[1] | |
| | + | - | Inadq[2] | + | - | + | - | + | - |
| **FFPE** | | | | | | | | | |
| **FISH+ (N=28)** | 16 (57.1) | 8 (28.6) | 4 (14.3) | 22(78.6) | 6 (21.4) | 23 (82.1) | 5(17.9) | 27 (96.4) | 1 (3.6) |
| **FISH- (N=2)** | 0 (0.0) | 2 (100.0) | 0 (0.0) | 1 (50.0) | 1 (50.0) | 0 (0.0) | 2 (100.0) | 1 (50.0) | 1 (50.0) |
| **Accuracy** | 18/24 (69.2) | | | 23/30 (76.7) | | 25/30 (83.3) | | 28/30 (93.3) | |
| **FFPE** | | | | | | | | | |
| **RT-PCR+ (N=16)** | - | - | - | 12 (75.0) | 4 (25.0) | 14 (87.5) | 2 (12.5) | 16 (100.0) | 0 (0.0) |
| **RT-PCR- (N=10)** | - | - | - | 7 (70.0) | 3 (30.0) | 6 (60.0) | 4 (40.0) | 8 (80.0) | 2 (20.0) |
| **Accuracy** | - | | | 15/26 (57.7) | | 18/26 (69.2) | | 18/26 (69.2) | |

[1]plasma or platelets; [2]inadequate.

Presented by CK Park, WCLC, KATRD and KALC 2017

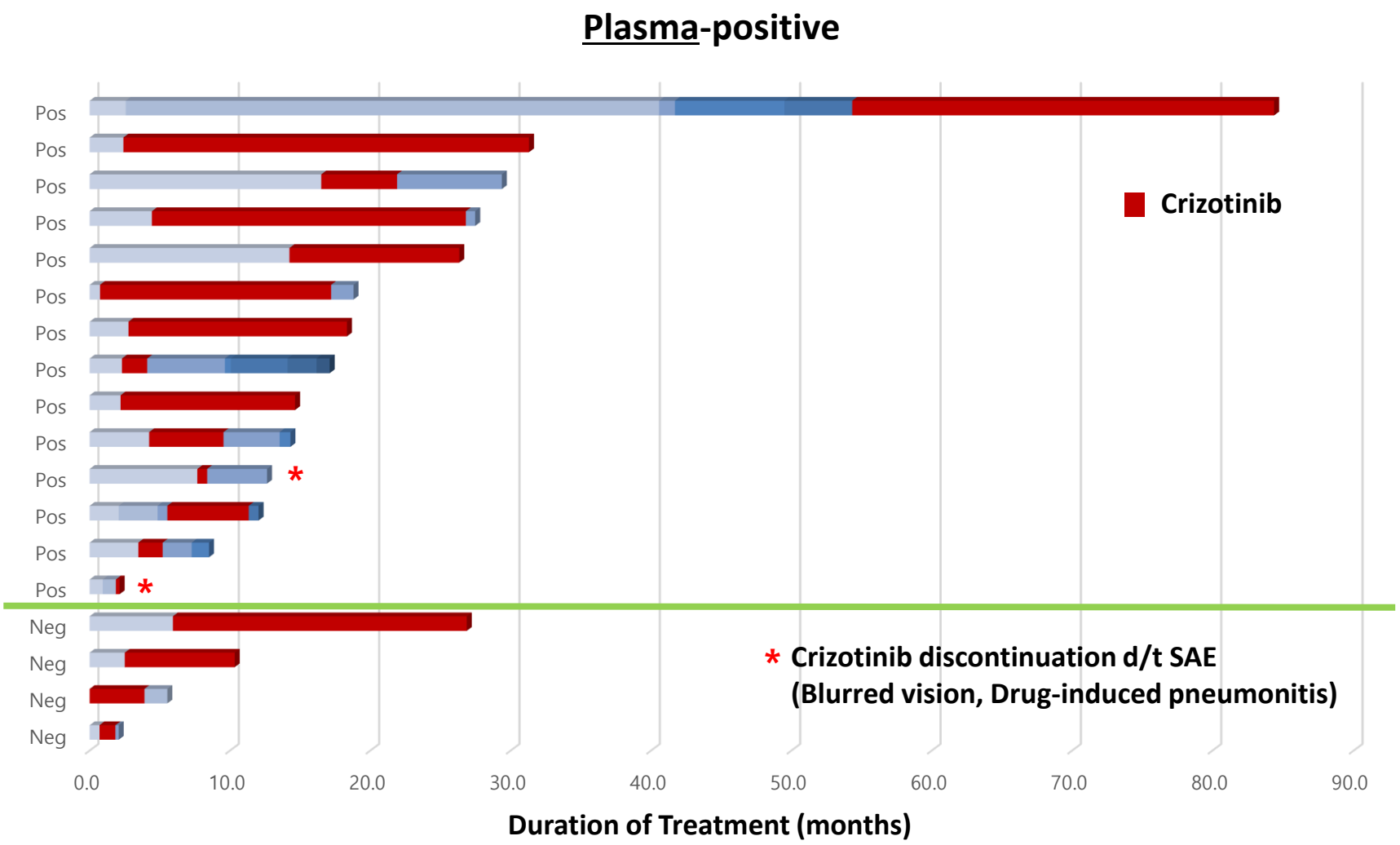
| Characteristics, n (%) | Total (N=30) | Plasma | | | Platelets | | |
|---|---|---|---|---|---|---|---|
| | | + (N=23) | - (N=7) | p | + (N=23) | - (N=7) | p |
| FISH result | | | | | | | |
| Proportion, %, median (IQR) | 27.5 (17.5-50.0) | 30.0 (20.0-50.0) | 15.0 (15.0-22.5) | 0.062 | 30.0 (20.0-50.0) | 20.0 (12.5-32.5) | 0.104 |
| EGFR mutation | 5 (16.7) | 4 (17.4) | 1 (14.3) | 1.000 | 4 (17.4) | 1 (14.3) | 1.000 |
| RNA, ng/ul, median (IQR) | | | | | | | |
| FFPE tissue | 26.4 (10.9-113.1) | - | - | - | - | - | - |
| Plasma | 2.4 (2.1-2.6) | 2.5 (2.1-2.8) | 2.4 (2.3-2.5) | 0.417 | - | - | - |
| Platelet | 2.5 (1.9-3.3) | - | - | - | 2.5 (2.1-3.4) | 1.7 (1.5-3.4) | 0.302 |
| Pemetrexed Tx | 20 (66.7) | 17 (73.9) | 3 (42.9) | 0.181 | 16 (69.6) | 4 (57.1) | 0.657 |
| Duration, months, median (IQR) | 2.8 (2.3-4.3) | 3.8 (2.4-14.5) | 5.8 (3.3-6.0) | 0.711 | 4.1 (2.6-12.6) | 4.1 (1.5-11.4) | 0.637 |
| Best response | | | | 0.353 | | | 0.862 |
| PR | 5 (25.0) | 4 (23.5) | 1 (33.3) | | 4 (25.0) | 1 (25.0) | |
| SD | 12 (60.0) | 11 (64.7) | 1 (33.3) | | 10 (62.5) | 2 (50.0) | |
| PD | 3 (15.0) | 2 (11.8) | 1 (33.3) | | 2 (12.5) | 1 (25.0) | |
| ORR | 5 (25.0) | 4 (23.5) | 1 (33.3) | 1.000 | 4 (25.0) | 1 (25.0) | 1.000 |
| DCR | 17 (85.0) | 15 (88.2) | 2 (66.7) | 0.404 | 14 (87.5) | 3 (75.0) | 0.509 |
| Crizotinib Tx | 18 (60.0) | 14 (60.9) | 4 (57.1) | 1.000 | 14 (60.9) | 4 (57.1) | 1.000 |
| Duration, months, median (IQR) | 8.5 (5.3-18.0) | 7.2 (1.9-14.7) | 3.2 (1.8-11.0) | 0.457 | 7.2 (4.0-18.0) | 1.5 (0.7-5.4) | 0.071 |
| Best response | | | | 0.717 | | | 0.100 |
| PR | 8 (44.4) | 7 (50.0) | 1 (25.0) | | 8 (57.1) | 0 (0.0) | |
| SD | 5 (27.8) | 3 (21.4) | 2 (50.0) | | 4 (28.6) | 1 (25.0) | |
| PD | 5 (27.8) | 4 (28.6) | 1 (25.0) | | 2 (14.3) | 3 (75.0) | |
| ORR | 8 (44.4) | 7 (50.0) | 1 (25.0) | 0.588 | 8 (57.1) | 0 (0.0) | 0.092 |
| DCR | 13 (72.2) | 10 (71.4) | 3 (75.0) | 1.000 | 12 (85.7) | 1 (25.0) | 0.044 |

ORR, objective response rate;

DCR, disease control rate.

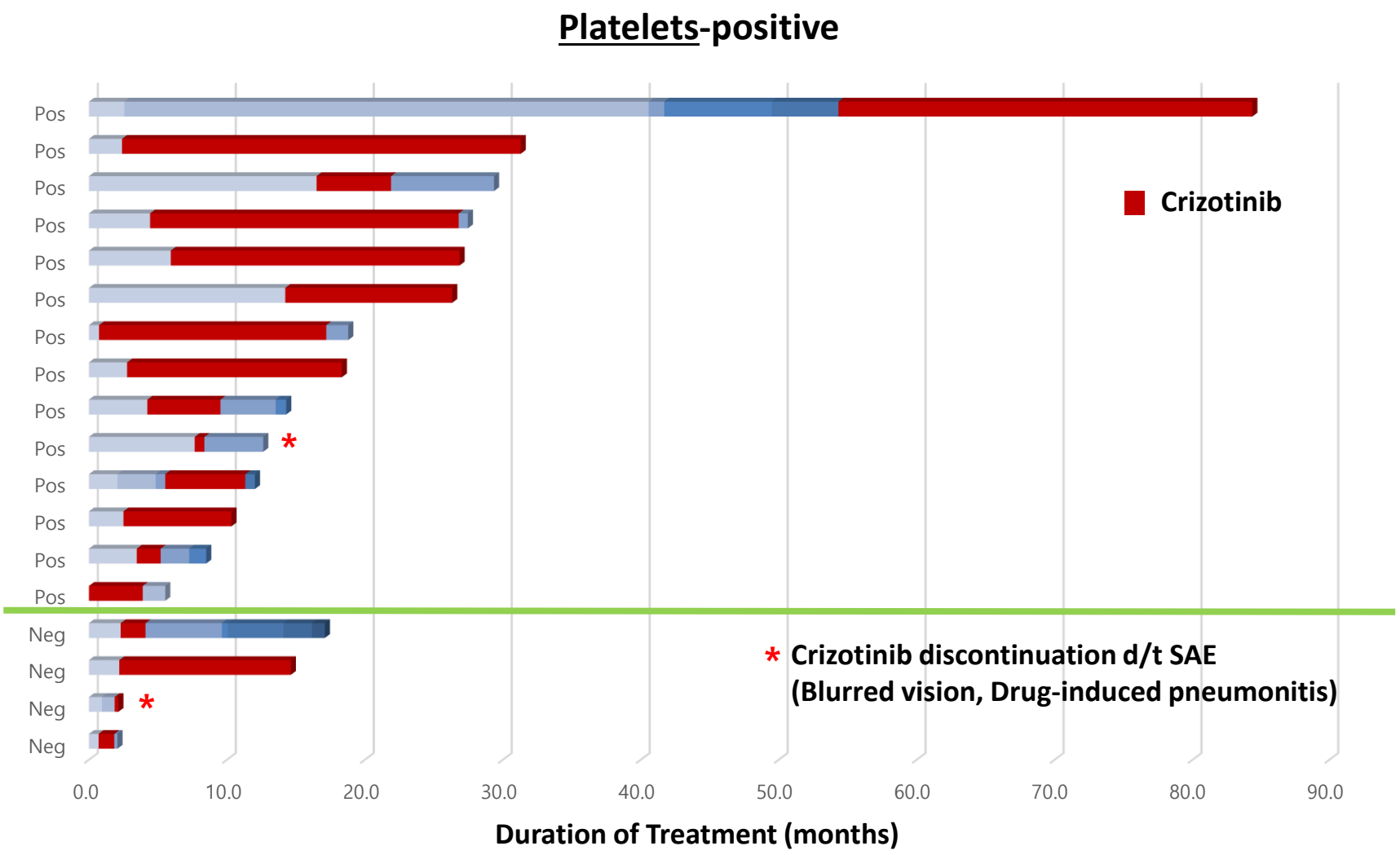
Presented by CK Park, WCLC, KATRD and KALC 2017

# Clinical Courses of ALK-positive NSCLC according to Detection of EML4-ALK by Liquid biopsy (N=18)




Presented by CK Park, WCLC, KATRD and KALC 2017

# Clinical Courses of ALK-positive NSCLC according to Detection of EML4-ALK by Liquid biopsy (N=18)




Presented by CK Park, WCLC, KATRD and KALC 2017

# Methods – Monitoring

- Whole blood sampling (10ml or more) on every visits
- RNA extraction & RT-PCR assay

  → Repeat regularly same as screening

- Clinical data collection until RECIST PD or EOT by investigators
- If positive conversion (liquid biopsy (-)→(+)), consider plasma PCR or NGS for ALK resistance mutation

  - search for available institutes or company

  - development of specific PCR kit for ALK resistance mutation : proposal

  - contract with sponsor (plasma NGS)

# Methods – Progression

- RECIST PD or EOT by investigators
- Tumor rebiopsy in available patients
- Consider tumor PCR or NGS for ALK resistance mutation

  - search for available institutes or company

  - development of specific PCR kit for ALK resistance mutation : proposal

  - contract with sponsor
- Compare with previous plasma PCR or NGS results for ALK resistance mutation
- 2nd line treatment according to ALK resistance mutation

  - If ALK mutation(+), Next generation ALK inhibitor

  - If ALK mutation(-), Cytotoxic chemotherapy

# Assessment

Diagnosis, Monitoring

- Detection rate of ALK fusion transcript for liquid biopsy at diagnosis or during treatment
  - Sensitivity and specificity of liquid biopsy compared with tumor FISH

Monitoring

- Objective response rate (ORR), assessed by RECIST v1.1
- Progression-free survival (PFS)
- Duration of response (DoR)
- Time to negative or positive conversion (TNC or TPC)
  - Interval from treatment start to confirmation of conversion

Progression

- Detection rate of ALK resistance mutation for liquid biopsy after positive conversion
  - Sensitivity of liquid biopsy compared with tumor rebiopsy at progression

# Treatment Regimen

- **1st line treatment**

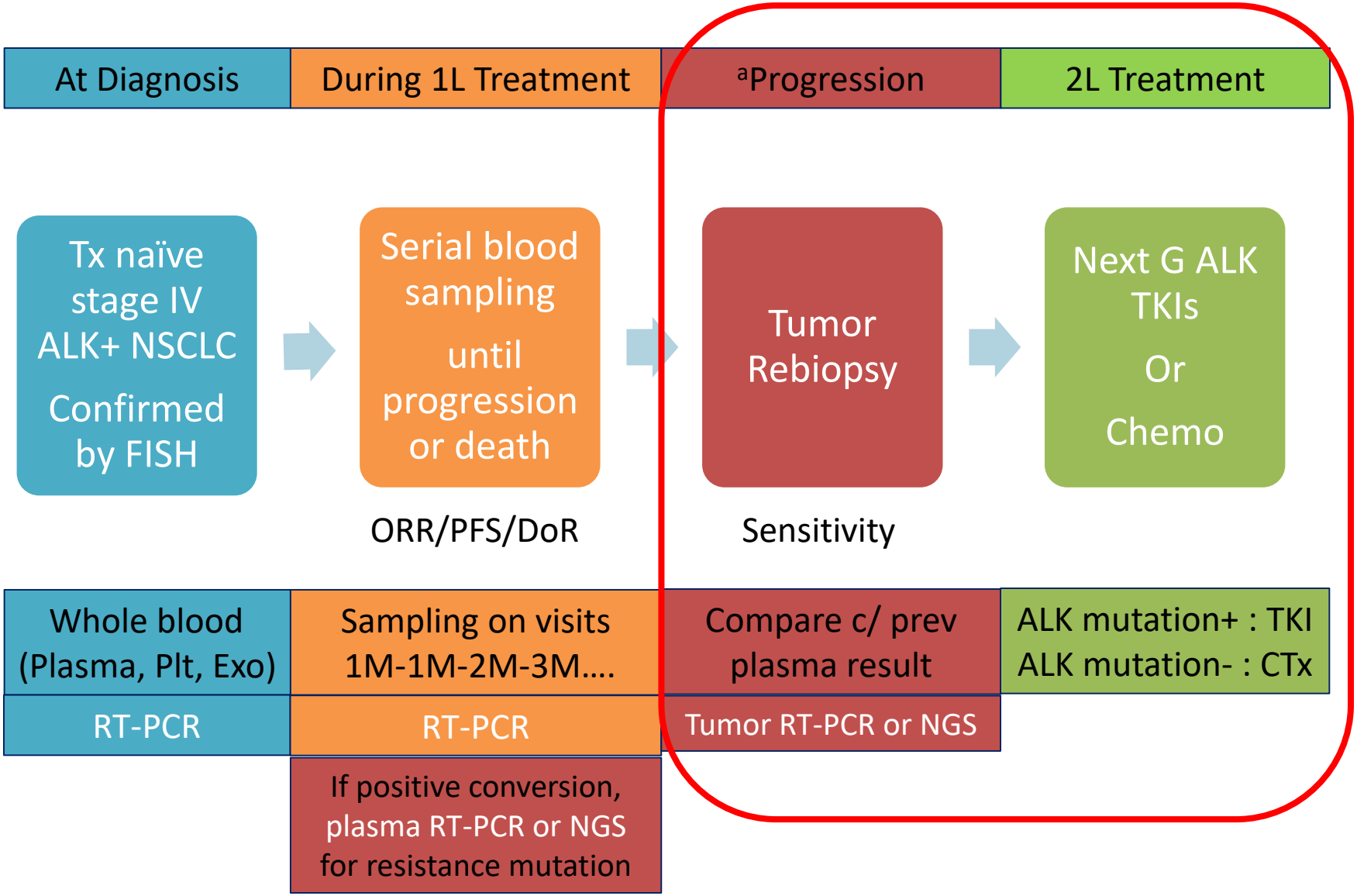
**1) Crizotinib (250mg bid)**

- **Pro** : SOC in ALK+ NSCLC, KFDA approved and reimbursable regimen
- **Con** : lower fraction of on-target resistance mutation than Next-gen ALK TKIs

**2) Alectinib (600mg bid)** or **Ceritinib (750mg qD s/ or 450mg qD c/ meal)**

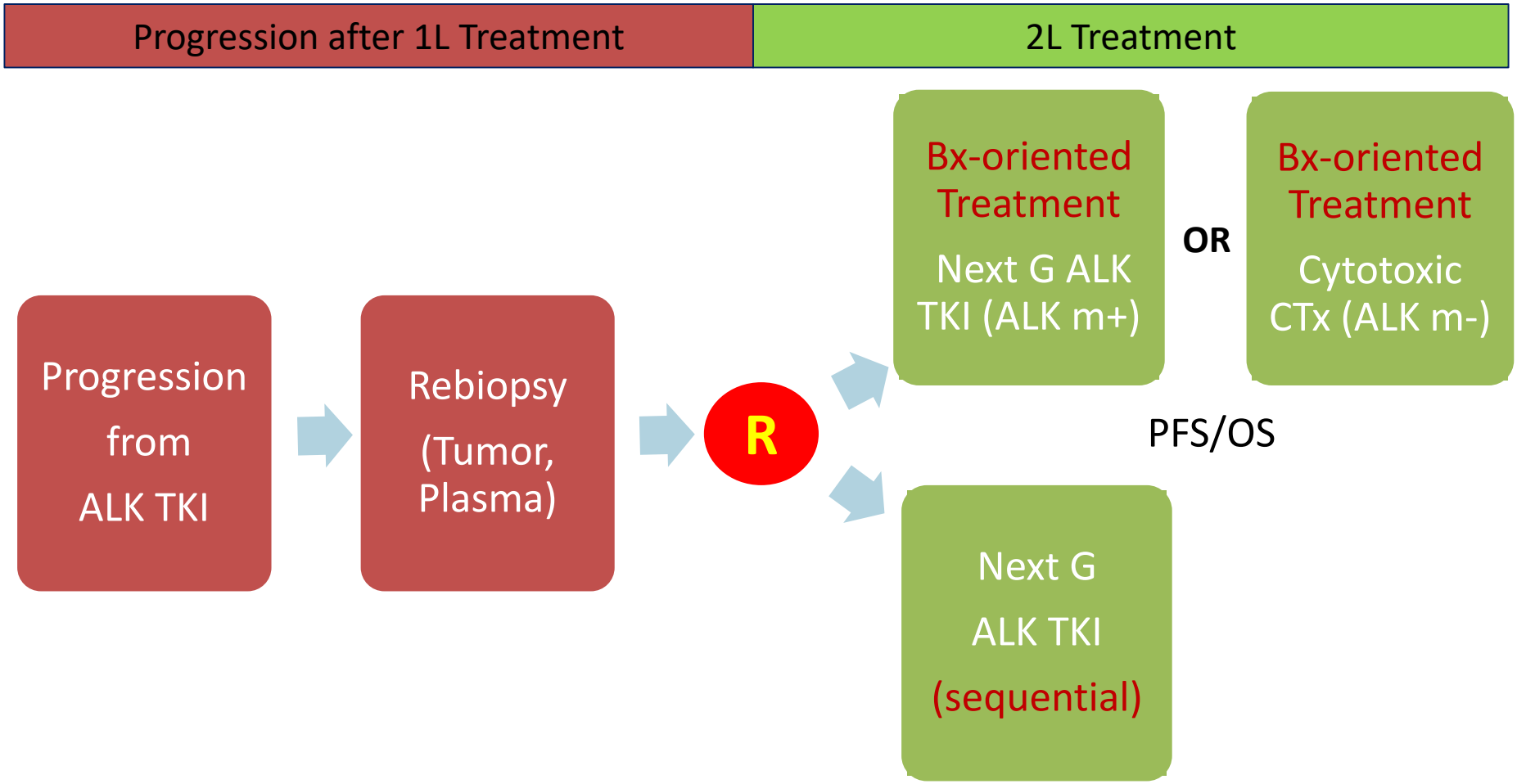
- **Pro** : Impressive front-line survival data, Good CNS effect, tolerable safety, Sponsor`s interest, higher fraction of on-target resistance mutation
- **Con** : KFDA not approved (alectinib) or not reimbursable (ceritinib) in 1L (needs for sponsorship), uneasy to access 2L ALK TKIs (Brigatinib, Lorlatinib)

# Future Direction




[a]according to RECIST v1.1 or investigator`s assessment

# Future Direction




Adapted from WCLC 2017

# Thank You for Your Attention

## Discussion

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