ALK rearrangements in lung cancer: Overview of science and therapeutic resistance mechanisms

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Disclosures

From June 2012 to present

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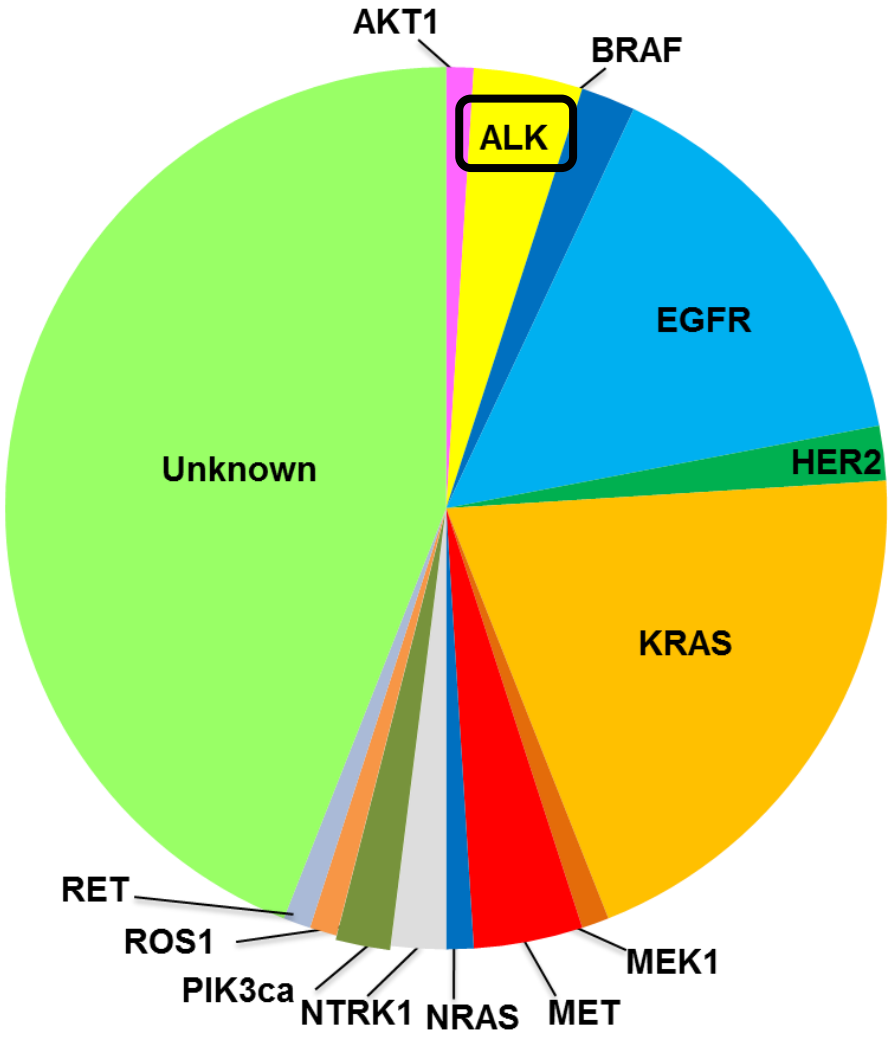
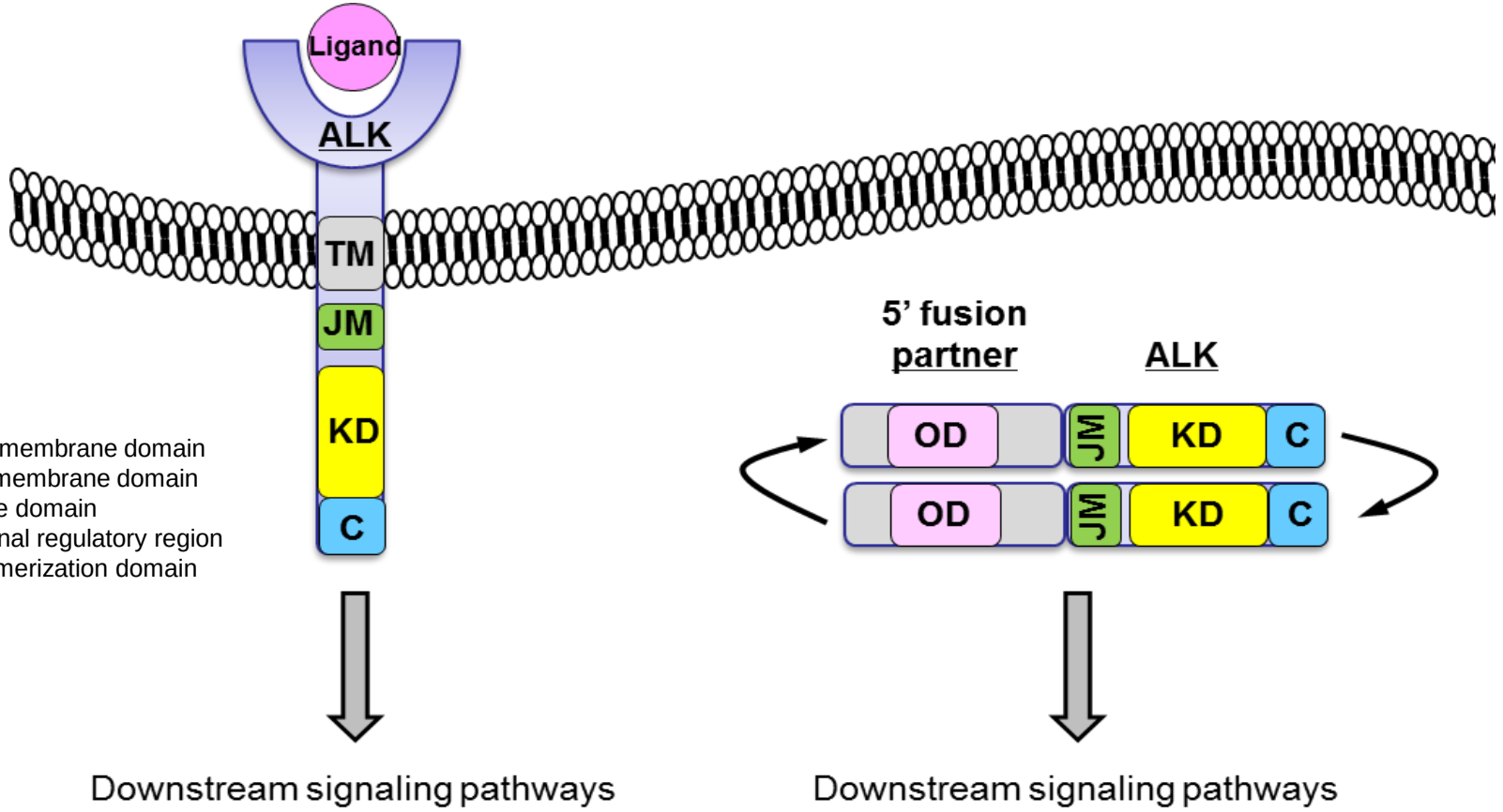
- Novartis, Astra Zeneca

Molecular Subsets of Lung Adenocarcinoma Defined by 'Driver' Mutations



Frequency of driver mutations in NSCLC	
AKT1	1%
ALK	3-7%
BRAF	1-3%
EGFR	10-35%
HER2	2-4%
KRAS	15-25%
MEK1	1%
MET	~4%
NRAS	1%
NTRK1	~3%
PIK3CA	1-3%
RET	1-2%
ROS1	1-2%

Comparison between ALK receptor and ALK fusion protein



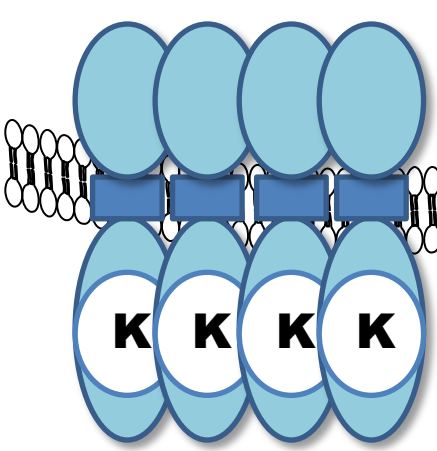
Key:
TM = Transmembrane domain
JM = Juxtamembrane domain
KD = Kinase domain
C = C terminal regulatory region
OD = Oligomerization domain

Notable differences:

- ALK fusion protein has lost: (1) extracellular/ligand binding domain, (2) transmembrane domain (in most cases).
- ALK fusion protein has gained: a fusion partner, which typically has an oligomerization domain (OD).
- Change in subcellular localization: ALK fusion proteins are typically cytoplasmic.

‘Driver’ mutations in ALK are found in a variety of different tumor types

ALK Amplification



- Neuroblastoma

ALK point mutations

- Neuroblastoma
- Anaplastic thyroid cancer

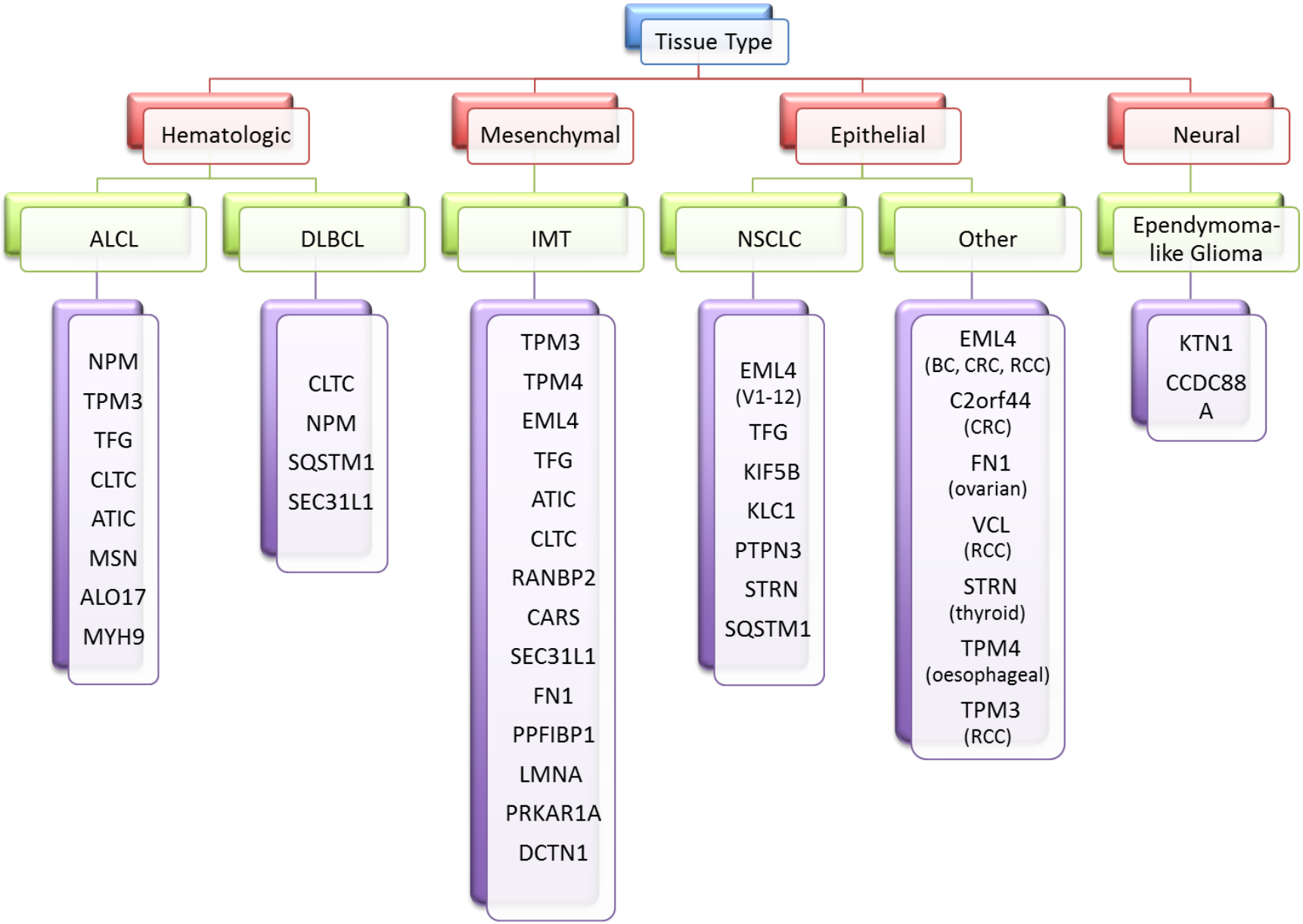
ALK Gene Fusion

- Lung Cancer
- Lymphoma (ALCL, DLBCL)
- Sarcoma (IMT)
- Case reports: breast cancer, colon cancer, ovarian cancer, kidney cancer, esophageal squamous carcinoma, renal cell carcinoma, renal medullary, thryoid, spitz tumors, AML, bladder cancer, rectal adenocarcinoma.

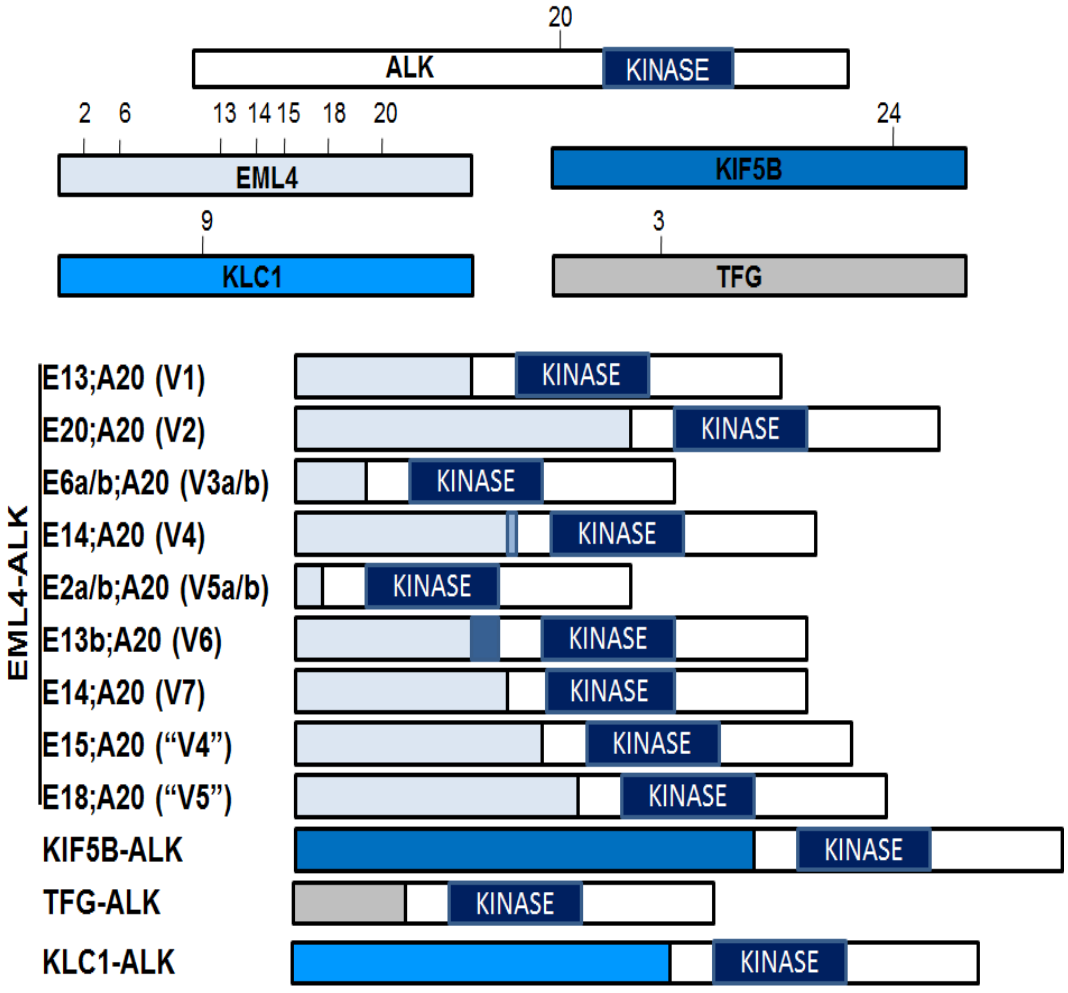
ALK-ATI

- Melanoma

Complex array of ALK fusion partners amongst different cancer types

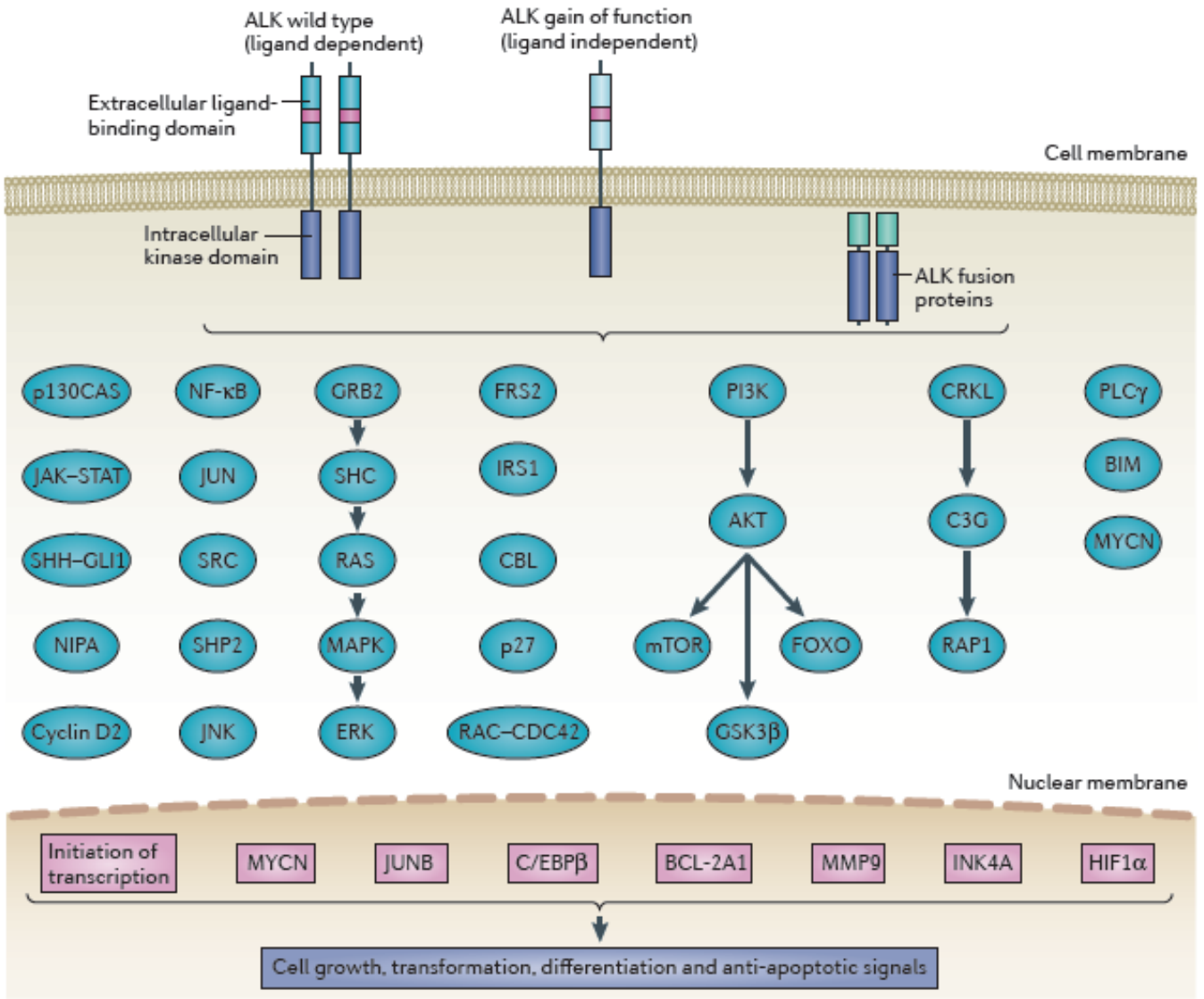


Schematic Representation of *ALK* Fusions in NSCLC



- All fusions contain the entire ALK tyrosine kinase domain.
- Multiple ALK fusions with various gene fusion partners have been described in NSCLC, most commonly EML4-ALK.
- The various 5' fusion partners promote dimerization and constitutive activation of ALK kinase activity resulting in a gain of function effect.
- *ALK* FISH and ALK IHC will not identify the gene fusion partner.

Signaling downstream of ALK



Hallberg Nature Reviews Cancer 2013

Crizotinib is a Standard Therapy for Patients with Metastatic ALK+ NSCLC

	PROFILE 1001[1] (N=143)	PROFILE 1005[2] (N=259)	PROFILE 1007[3] (N=172)	PROFILE 1014[4] (N=172)
Phase	1	2	3	3
Line of therapy	Any line	2nd line and beyond	2nd line	1st line
Response rate	61%	60%	65%	74%
PFS, median (mos)	9.7	8.1	7.7	10.9
Survival probability at 12 mos	75%	NA	70%	84%

1. Camidge DR, et al. *Lancet Oncol* 2012;13(10):1011–9;
2. Kim DW, et al. ASCO 2012; Abstr 7533;
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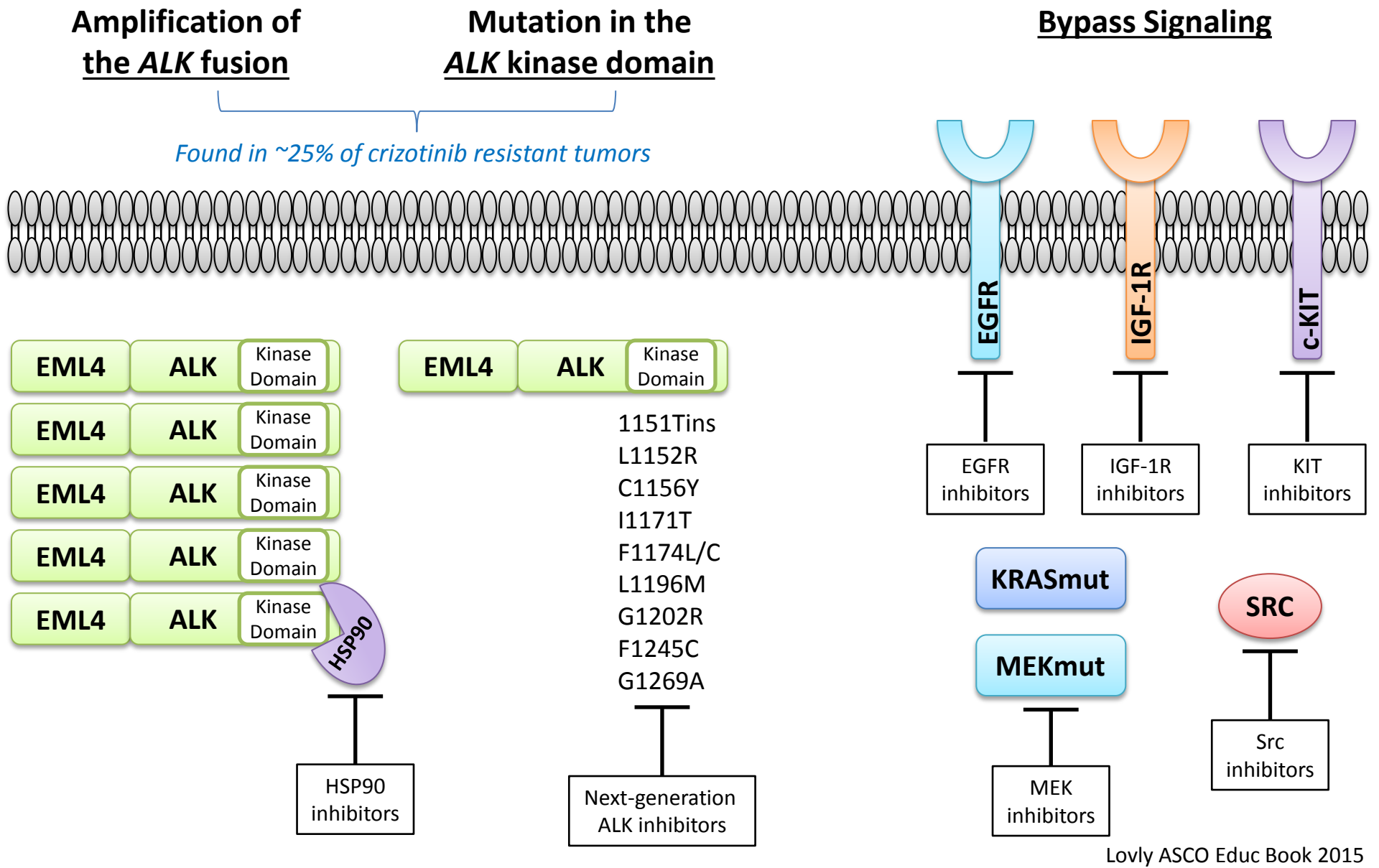
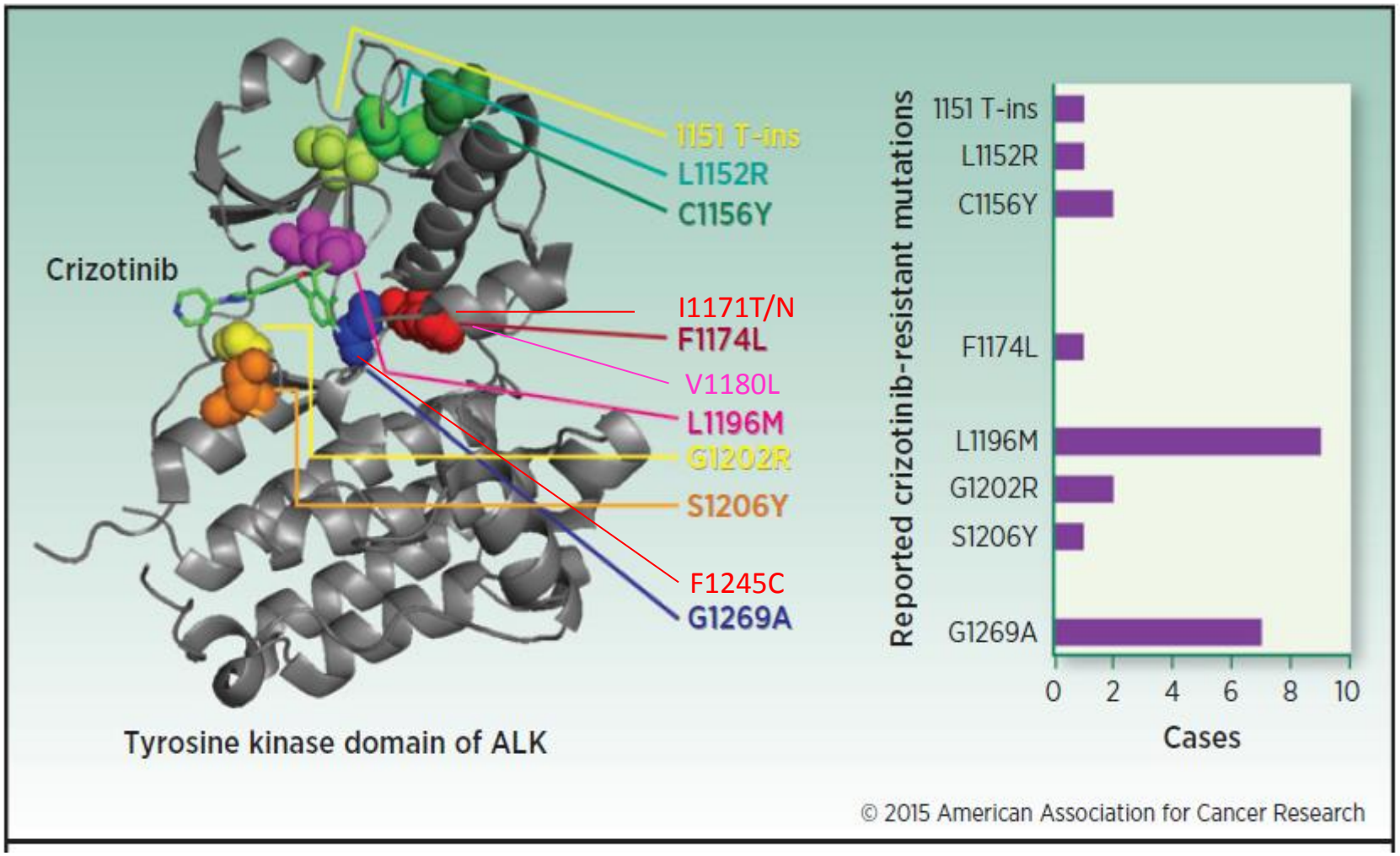
Slide courtesy of Alice Shaw

Mechanisms and potential strategies to overcome acquired resistance to crizotinib



Lovly ASCO Educ Book 2015

Crizotinib Resistance Mutations



Adapted from:
Katayama, Lovly, Shaw 2015 Clin Can Res
Awad and Shaw 2014 Clin Adv Hematol Oncol
Kodityal 2016 Lung Cancer

‘NEXT-GENERATION’ ALK INHIBITORS

TKI	COMPANY	OTHER TARGETS	STATUS
Alectinib (CH5424802)	Genentech/ Roche	LTK	FDA accelerated approval for the treatment of people with ALK+ metastatic NSCLC who have progressed on or are intolerant to crizotinib, 12/2015
Brigatinib (AP26113)	Ariad	ROS1	Breakthrough therapy designation from the FDA for the treatment of people with ALK+ metastatic NSCLC who have progressed on or are intolerant to crizotinib, 10/2014
Ceritinib (LDK378)	Novartis	IGF-1R, IR	FDA accelerated approval for the treatment of people with ALK+ metastatic NSCLC who have progressed on or are intolerant to crizotinib, 4/2014
Entrectinib (RXDX-101)	Ignyta	Trk, ROS1	Phase 1/2
Lorlatinib (PF-06463922)	Pfizer	ROS1	Phase 1/2
TSR-011	Tesaro	Trk	Phase 1/2
X-396	Xcovery	MET	Phase 1/2

Adapted from Awad and Shaw 2014 Clin Adv Hematol Oncol

What do we know about resistance to next generation ALK TKIs?

Ceritinib Resistance

in silico and in vitro studies:

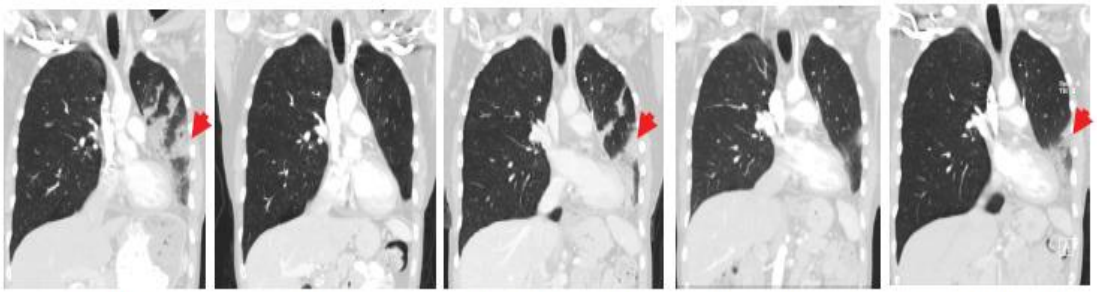
- Ceritinib inhibits ALK L1196M, G1269A, I1171T and S1206Y mutations
- Ceritinib does not overcome two crizotinib-resistant ALK mutations, G1202R and F1174C/V

Patient Id	EML4-ALK sequence at Crizotinib Resistance	EML4-ALK sequence at Ceritinib Resistance
MGH011	S1206Y	G1202R
MGH015	WT	WT
MGH023	WT	F1174C
MGH034	WT	WT
MGH049	WT	WT
MGH051	WT	G1202R
MGH057	N/A	WT
MGH061	WT	WT
JFCR013	N/A	WT
JFCR021	G1269A (right lung)	F1174V (left lung) and G1202R (right lung)

2/10 pts 4/10 pts

Resistance mutations

MGH011 Lung CT scan



Baseline | After 8 weeks of crizotinib | After 34 months of crizotinib | After 12 weeks of Ceritinib | After 15 months of Ceritinib

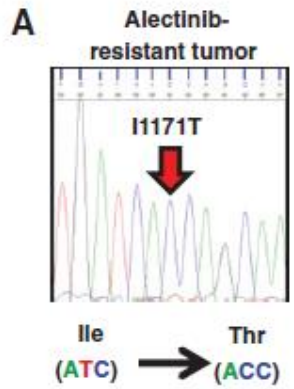
EML4-ALK sequence: WT S1206Y G1202R

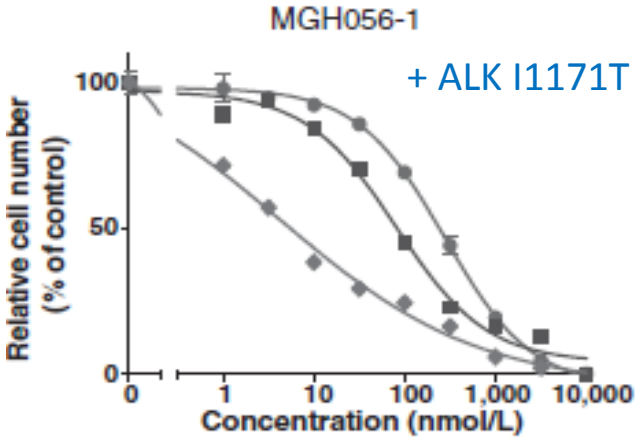
Friboulet Cancer Discovery 2014

Alectinib Resistance

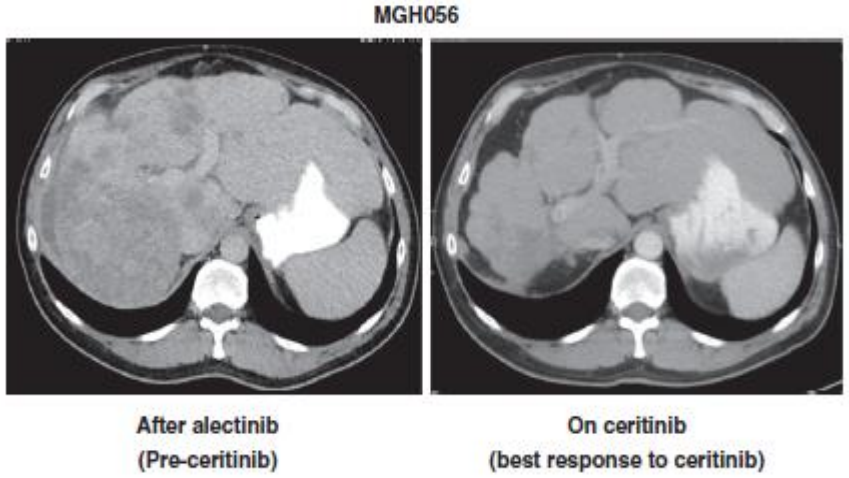
Case Report:

- Patient with advanced ALK-positive NSCLC
- Previously received three lines of chemotherapy
- Followed by crizotinib with PR x 8 months
- Followed by alectinib with PR x 4 months
- Biopsy of alectinib resistant tumor
- Cell line generated from the resistant tumor sample (MGH056-1)
- ALK I1171T mutation identified
- In vitro studies showed this mutation was resistant to crizotinib and alectinib but not to ceritinib.





Drug	IC_{50} (nmol/L)
Crizotinib	236 nmol/L
Alectinib	80 nmol/L
Ceritinib	4.3 nmol/L



Also identified V1180L mutation from a cell line model.

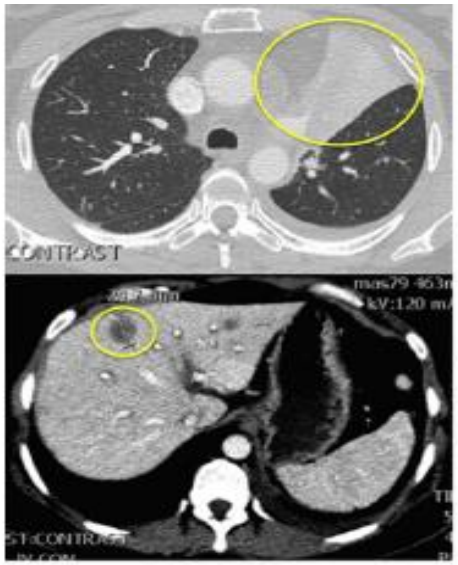
- Like I1171T, this mutation conferred resistance to alectinib and crizotinib, but was sensitive to ceritinib.

Katayama CCR 2014

Alectinib Resistance

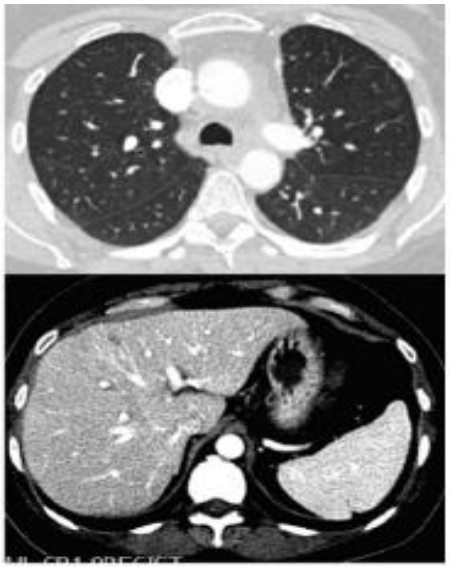
Case Report:

- Patient with advanced ALK-positive NSCLC
- Received crizotinib, developed grade 3 transaminitis
- Switched to alectinib, PR achieved within 3 months
- At 5 months, the patient developed jaundice and was found to have several new liver metastases
- Biopsy of alectinib resistant tumor → ALK I1171N mutation identified
- Patient started on certinib with PR



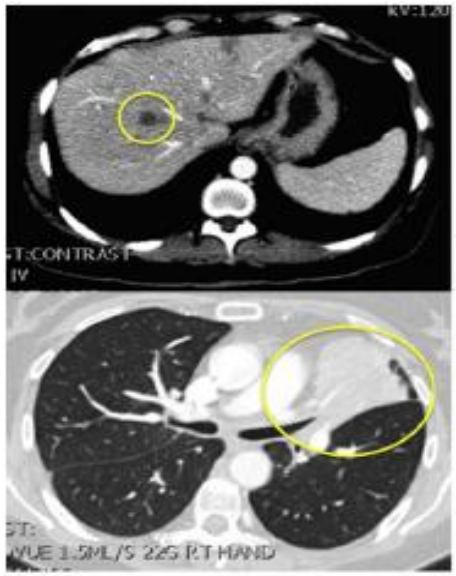
C 4 days before alectinib

Before alectinib



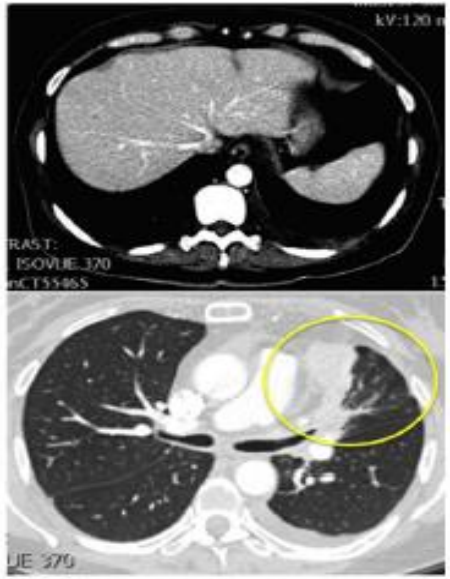
D 3 months after alectinib

Response to alectinib



At time of progression from alectinib and 13 days prior to ceritinib

Resistance to alectinib



6 weeks after ceritinib

Response to ceritinib

Ou Lung Cancer 2015

MET amplification at the time of acquired resistance to alectinib

Case Report

- Patient with *ALK*-rearranged NSCLC
- 3 regimens of chemotherapy
- Alectinib (CH5424802): PR (-78%), 10.5 months
- Rapid progression, DIC
- Biopsy of liver lesion: *MET* amplification, no ALK kinase domain mutations
- Crizotinib, 5 months

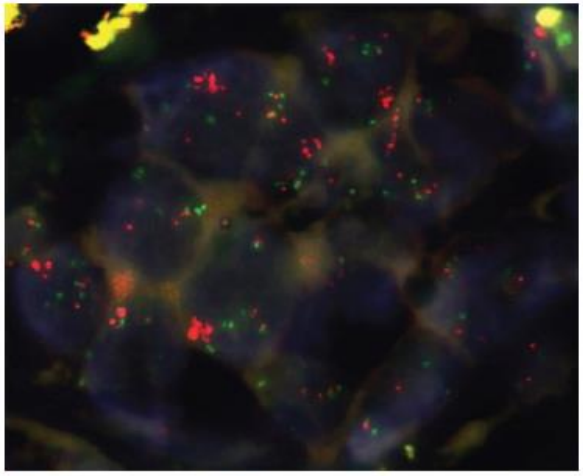
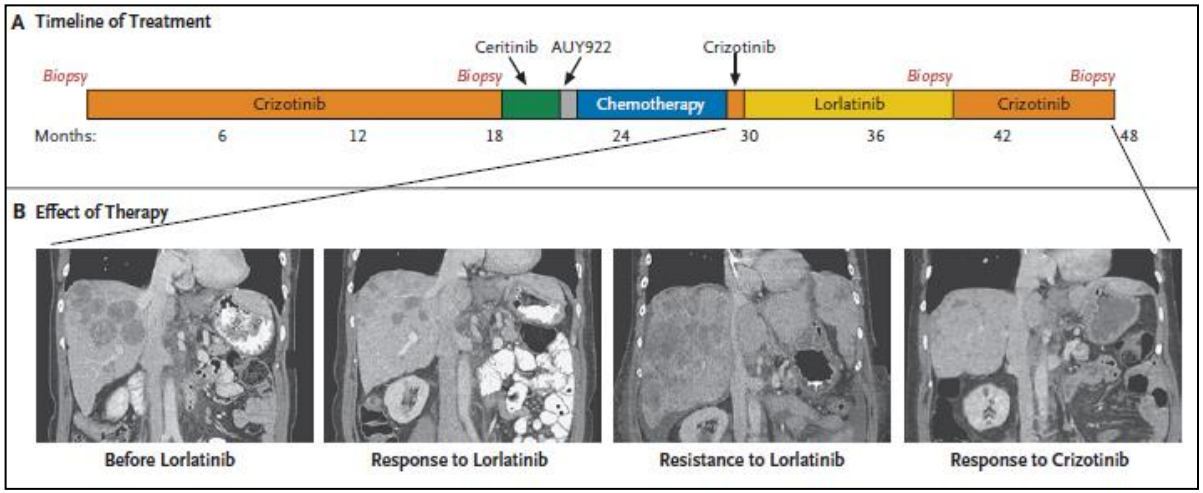
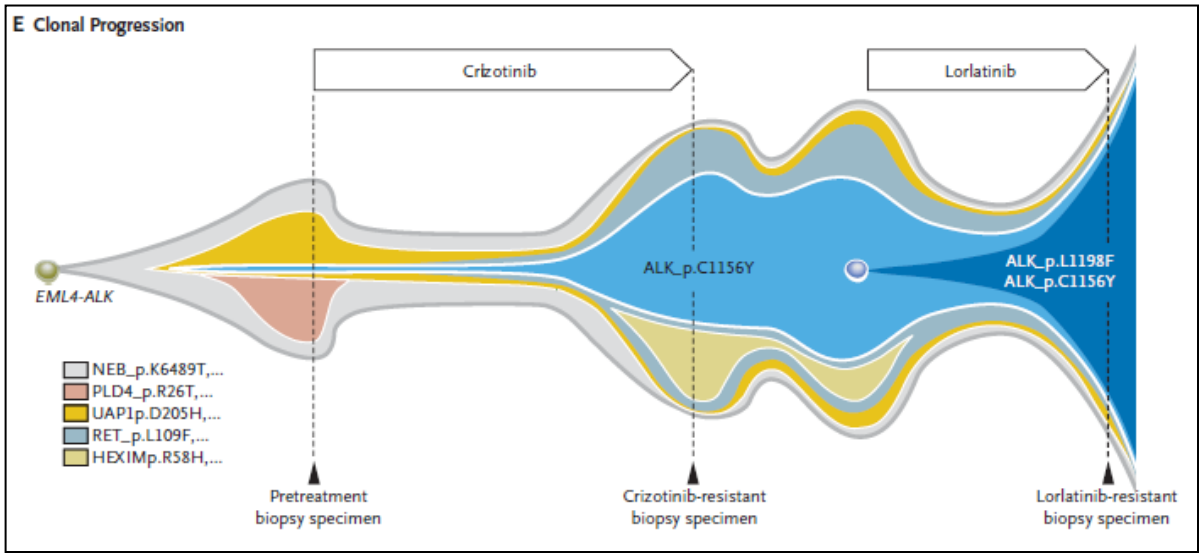


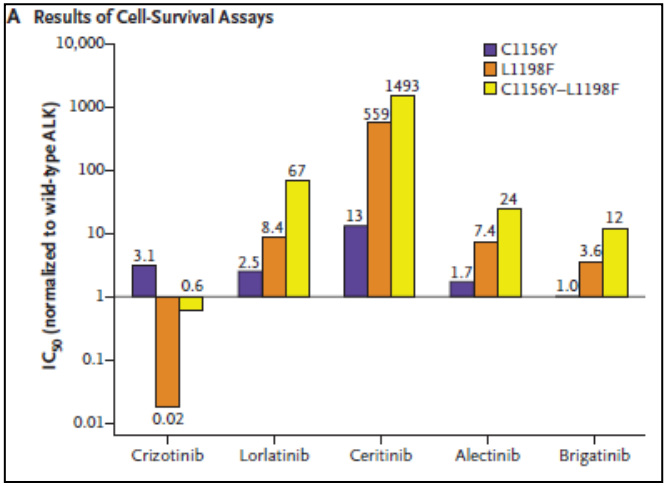
FIGURE 1. A fluorescence in situ hybridization assay showing the amplification of the *MET* gene. Blue: 4′,6-diamidino-2-phenylindole (DAPI); red: met proto-oncogene (*MET*); green: 7q11.21.

Gouji JTO 2014

Lorlatinib Resistance

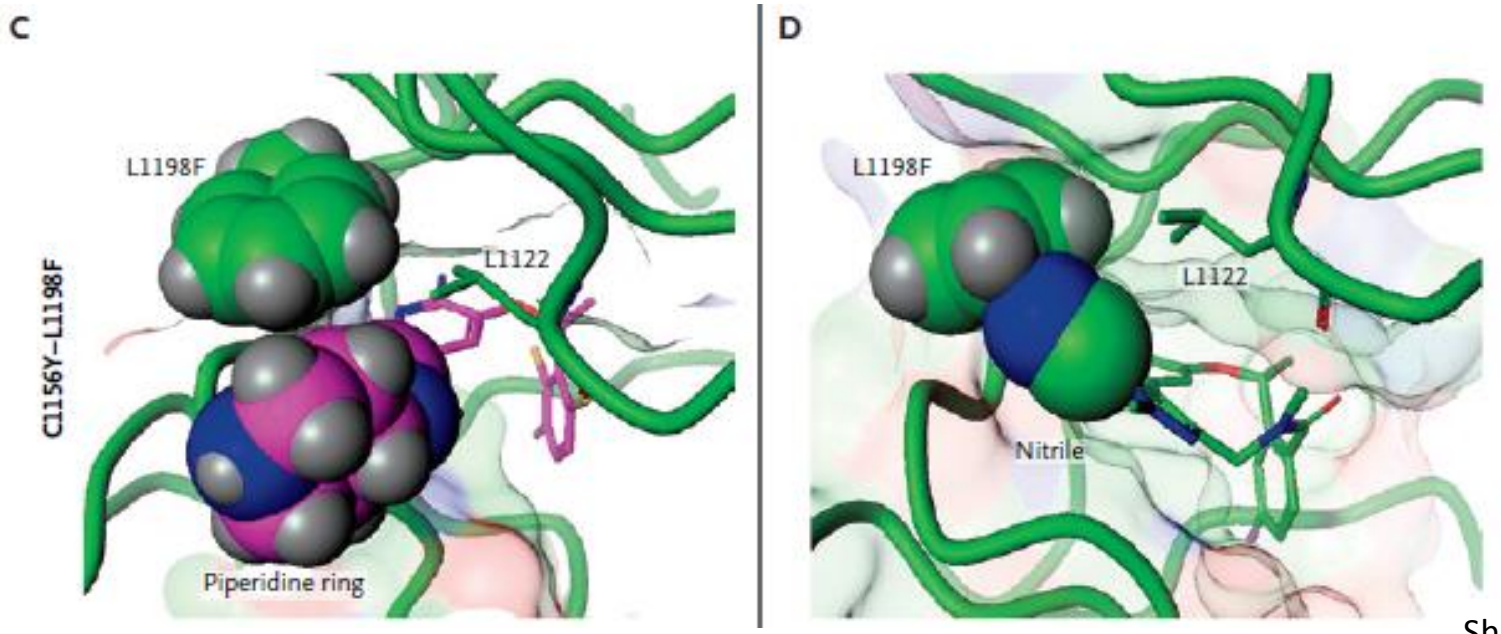






Shaw NEJM 2016

Lorlatinib Resistance



Shaw NEJM 2016

Crizotinib

Lorlatinib

ALK L1198 residue was targeted for lorlatinib's design to increase selectivity against the majority of other kinases which do not have a leucine residue at this position within the ATP binding pocket. [Ref: Johnson T Journal of Medicinal Chemistry 2014]

ALK kinase domain mutations – drug efficacy

	1st gen	2nd gen			3rd gen
	Crizotinib	**Alectinib**	**Brigatinib**	**Ceritinib**	**Lorlatinib**
G1123S	Res	Sens[2]	N/D	Res[2]	N/D
1151Tins	Res	Res[3]	N/D	Res[7]	Sens[9]
L1152P/R	Res	Sens	N/D	Res[7]	Sens[9]
C1156Y/T	Res	Sens	N/D	Res[7]	Sens[9]
I1171T/N	Res	Res[4,5]	N/D	Sens[4,5,7]	N/D
F1174C/L/V	Res	Sens	Sens[6]	Res[7]	Sens[9]
V1180L	Res	Res [4]	N/D	Sens[4]	N/D
L1196M	Res	Sens[3]	Sens[6]	Sens[7]	Sens[9]
L1198F	Sens [1]	Res[1]	Res[1]	Res[1]	Res[1]
G1202R	Res	Res[3]	N/D	Res[7]	Sens[9]
S1206C/Y	Res	Sens[3]	Res[6]	Sens[7]	Sens[9]
F1245C	Res[8]	N/D	N/D	Sens[8]	N/D
G1269A/S	Res	Sens	N/D	Sens[7]	Sens[9]

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2. Toyokawa JTO 2015
3. Katayama STM 2012
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8. Kodityal Lung Cancer 2016
9. Zou Cancer Cell 2015
10. Bayliss Cel Mol Lif Sci 2015

How should we sequence therapy using second-generation ALK inhibitors?

- Duruisseaux M WCLC 2015 Abstract # 1355: study results suggest that treatment with a second-generation ALK TKI after crizotinib failure provides a survival advantage compared to crizotinib rechallenge or standard chemotherapy.
  - Median OS= 34.7 months vs. 19.6 months vs. 15.3 months

- The mutation(s) that develop on therapy will be important for selecting the next ALK inhibitor prescribed for the patient.

- NCI ALK Master Protocol was hoping to address this question. Stay tuned…

Conclusions, Future Directions, and Ongoing Questions

1) Multiple second-generation ALK inhibitors being developed.
   - ✓ High response rates across the board in crizotinib resistant tumors.

2) Optimal sequence of these ALK inhibitors is currently unknown.
   - ✓ The presence of specific ALK kinase domain mutations associated with resistance will also matter in selecting therapy.
   - ✓ AEs may dictate use of specific agents in specific clinical contexts.

3) Will crizotinib be replaced as the first-line ALK TKI of choice for patients with metastatic disease?

4) Will crizotinib be effective in the adjuvant setting?

5) How will immune therapy enter into the treatment of patients with ALK+ lung cancer?

6) Does the ALK fusion variant present matter prognostically or therapeutically?

Thank you!

Extra Slides

Brigatinib Resistance

2 brigatinib (AP26113)-resistant cell lines

-- both *NPM-ALK* lymphoma cell lines

-- One cell line (KARPAS-299) had NPM-ALK overexpression.

-- One cell line (SPU-M2) developed point mutations in the ALK kinase domain.

Table 3. Mutations reported by direct sequencing

Cell line	Mutation	Substitution
SUP-M2AR500A	4472T>G+4544C>T	F1174V+L1198F
SUP-M2AR500B	4316C>G+4538C>A	L1122V+L1196M
SUP-M2AR500C	4538C>A	L1196M
SUP-M2AR500D	4569C>G	S1206C

Table 4. IC_{50} values obtained by proliferation assay for each Ba/F3 NPM-ALK WT or mutagenized cell line are summarized

IC_{50} (μmol/L)	AP26113	CRIZOTINIB	CH5424802	LDK-378	ASP3026
WT	0.01165	0.1243	0.02911	0.0433	0.07159
L1122V	0.09735	0.3229	0.1548	0.3933	0.3493
P1139S	0.01798	0.1315	0.01881	0.1494	0.1333
F1174V	0.01787	0.1182	0.1082	0.04105	0.2831
L1196M	0.02491	0.4224	0.08548	0.04576	0.3552
L1198F	0.06797	0.01249	0.3503	0.9623	0.3849
S1206C	0.166	0.5337	0.1645	0.1785	1.227
L1122V+L1196M	0.7582	0.945	0.5955	0.3762	1.85
F1174V+L1198F	0.1421	0.005771	0.2628	0.4325	0.4161
L1196M+D1203N	0.3863	0.7426	0.1226	0.1292	0.1187

Ceccon 2014 Mol Cancer Res