Detectie en relevantie van fusiegenen

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Disclosures

Advisory board	:	Janssen, Merck, Roche
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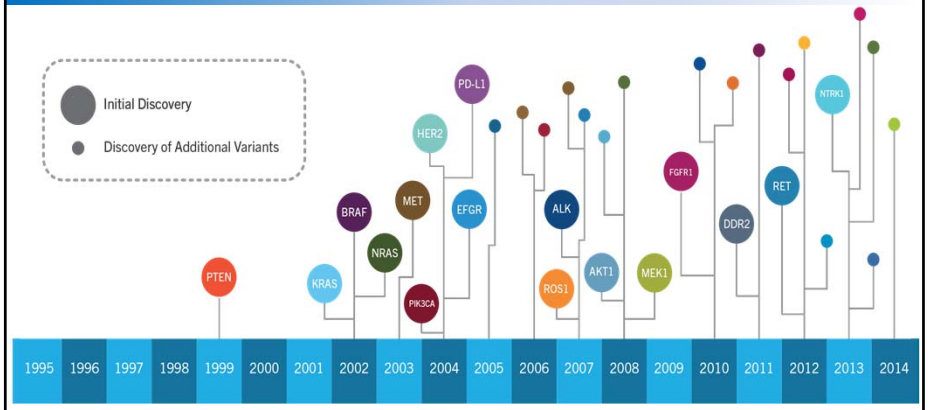
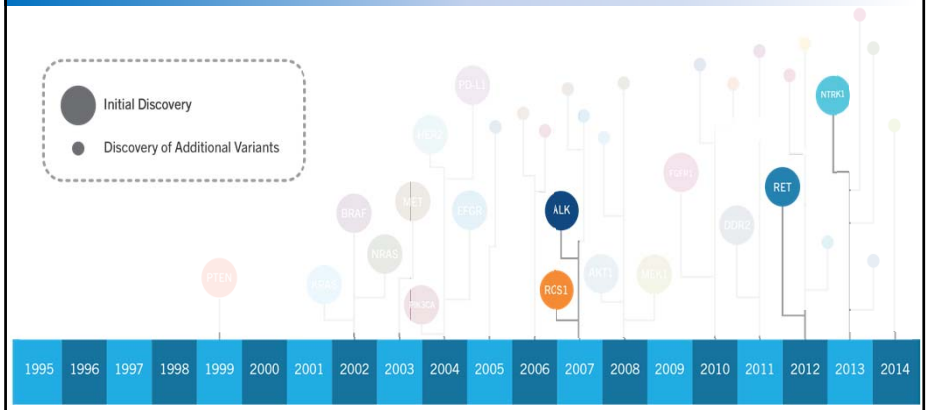
Lung cancer (2002-2014) : An explosion of bio(marker) diversity



www.thelungcancerproject.org

Lung cancer (2002-2014) : An explosion of bio(marker) diversity



www.thelungcancerproject.org

Molecular diagnostics of NSCLC

Next generation sequencing: SNV, indels and CNV

EGFR (12%) Erlotinib, Gefitinib, Afatinib, Osimertinib
BRAF (3%) Dabrafinib
MET (3%) Crizotinib, Capmatinib, Tepotinib
ERBB2 (3%) Trastuzumab, Afatinib
KRAS (25%) Ph 1/2: p.G12D-specific inihibitor BI-2852, pG12C-specific inhibitor MRTX849, and others
NRAS (<1%) none

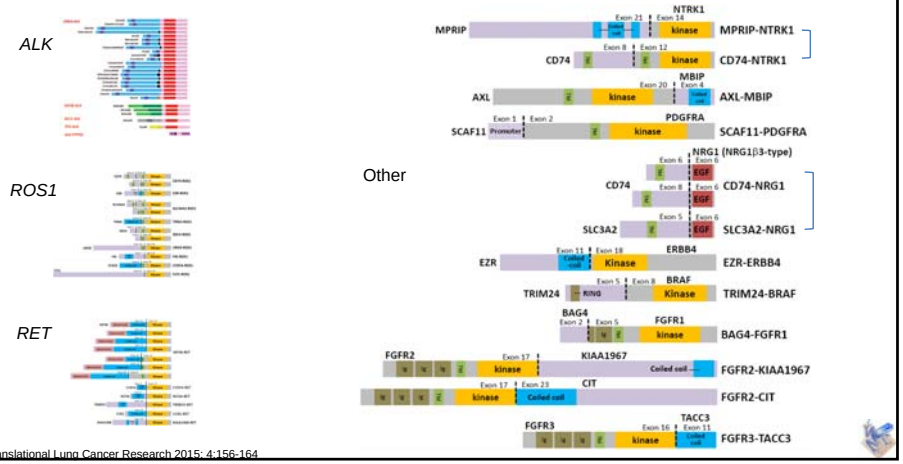
Gene fusion events

ALK (4%) Crizotinib, Ceritinib, Alectinib
ROS1 (2%) Crizotinib, Ceritinib
RET (1%) Alectinib, Blu-667 (phase II)
NTRK1 (0.2%) Entrectinib, Larotrectinib
NRG1 (0.2%) Afatinib
BRAF (0.2%) MEKi (preclinical), RAFi (preclinical)

ALK, *ROS1* and *RET* gene fusions in NSCLC

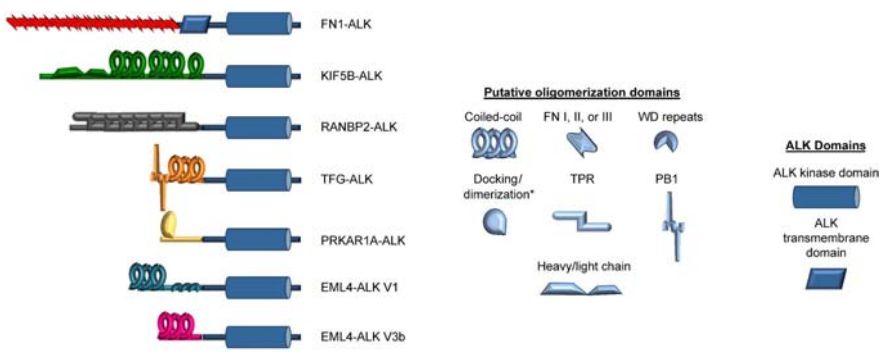
Translational Lung Cancer Research 2015; 4:156-164

Gene fusions in NSCLC



Translational Lung Cancer Research 2015; 4:156-164

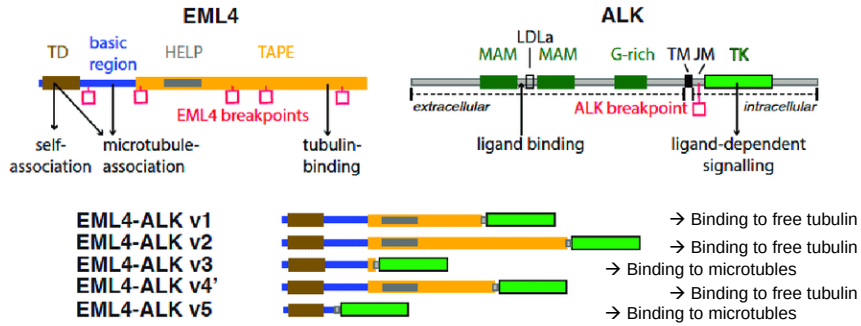
ALK fusions NSCLC



Differential subcellular localization controlled by the N-terminal fusion partner regulates the oncogenic mechanisms and output of RTK fusion oncoproteins.

Molecular Cancer Research 2018; 16:1724-1736

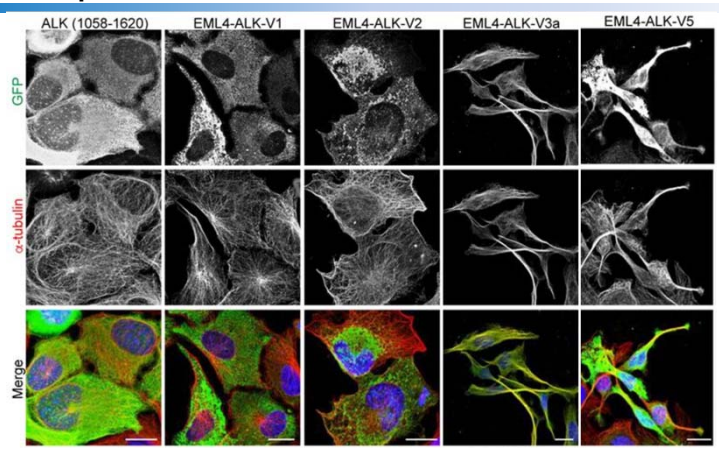
EML4-ALK fusions : not all are equal



Cancers 2017; 9, pii: E118

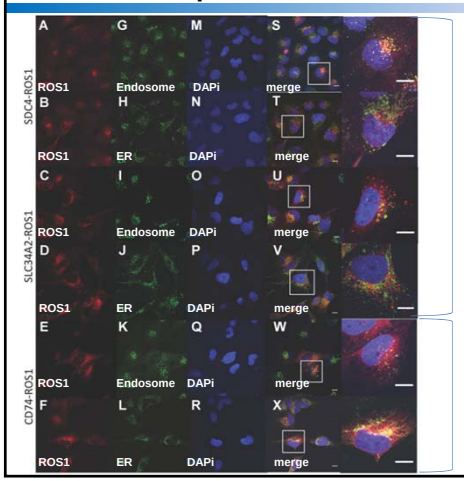
ALK fusion partner determines subcellular localisation

U2OS cell line



bioRXiv https://doi.org/10.1101/567305

ROS1 fusion partner determines subcellular localisation



SDC4-ROS1 and SLC34A2-ROS1 co-localize with endosomal markers and activates MAPK signaling

CD74-ROS1 colocalizes with ER marker, does not substantially activate MAPK signaling

Cancer Res 2019; 79: 546-566

Fusion transcript: does the partner matter clinically?

ALK

YES: Noh et al. *J Pathol*, 2017; 243:307-319 (n=46)
- ❑ EML4- ALK variant 3 correlates with more advanced stage and frequent metastases than variant 1
- ❑ Variant 3 is associated with shorter PFS (crizotinib) than variant 1
- ❑ More often crizotinib-associated resistance mutations in ALK variant 3

YES: Lin et al. *JCO*, 2018; 36:1199-1206
- ❑ Longer PFS with lorlatinib in EML4-ALK variant 3 than variant 1 (n=12)
- ❑ Crizotinib-resistant mutations are more common in EML4-ALK variant 3 than in variant 1 (n=106 and n=77)

YES: Li et al. *Lung Cancer,* 2018; 118:128-133 (n=60)
- ❑ EML4-ALK variant 2 is associated with longer PFS (crizotinib).
- ❑ EML4-ALK variant 3 correlates with a shorter duration of response to crizotinib

NO: Mitiushkina et al. *Biochimi,* 2018; 154:9-24 (n=64)
- ❑ Distinct EML4-ALK translocation variants (short v3/v5 vs long v2/v4) render similar clinical responsiveness to crizotinib and ceritinib

ROS1

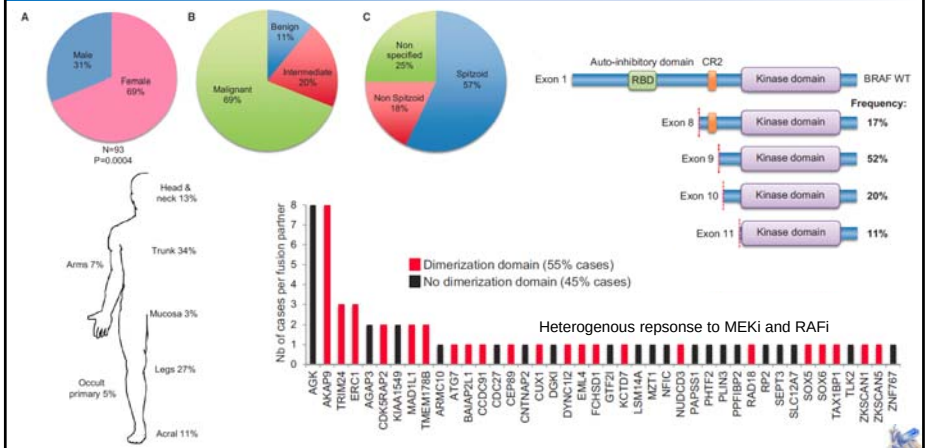
YES: Li et al. *J Thorac Oncol*, 2018; 7:987-995 (n=36)
- ❑ Pts with non-CD74 ROS1 fusion partners had a significant longer PFS (p=0.048) and OS (p=0.036)
- ❑ Pts with CD74-ROS1 are more likely to present with brain metastases (p=0.020)

NO: Patil et al. *J Thorac Oncol*, 2018; 13:1717-1726 (n=17)
- ❑ Pts with CD74-ROS1 do not more often present with brain metastases (p=0.620)

NO: Zhang et al. *Oncotarget*, 2016; 7:75145-75154 (n=13)
- ❑ Comparable PFS among pts with SCL34A2, EZR and CD74 ROS1 translocation

NO: He et al. *Oncol Res*, 2019; 27:901-910 (n=38)
- ❑ Comparable OS and PFS between CD74 vs non-CD74 ROS1 translocation pts upon Crizotinib treatment

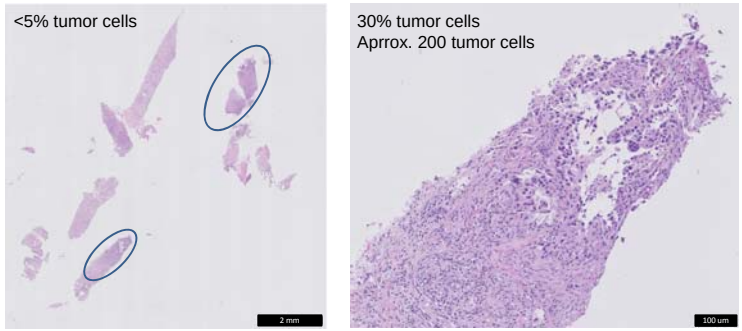
BRAF fusion transcripts in melanoma



Cell Reports 2019; 29:573-588

Tissue is an issue

Representative NSCLC biopsy



Problem: Consecutive FISH/IHC for *ROS1*, *RET*, *NTRK1, NRG1, MET*, *ERBB2* after ALK and PDL1 IHC, and mutation analysis.

➔ Multiplex targeted analysis of fusion gene transcript expression in FFPE

PATH fusion gene analysis - round robin

Purpose
- Inventory of technologies that are used for fusion gene detection
- Assessment of interlaboratory reproducibility of fusion gene detection
- Comparison of fusion gene reporting

Netherlands Cancer Institute · umcg · LUMC Leiden University Medical Center · Radboudumc · Amsterdam UMC · Erasmus MC · Maastricht UMC+

PATH

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RNA isolation method	Fusion transcript detection method
Promega® : ReliaPrep FFPE kit	ArcherDX® Fusionplex LUNG
Promega® : Maxwell RSC RNA FFPE Kit	ArcherDX® Fusionplex CTL
Siemens® : Versant TPS, TNA FFPE kit	Asuragen® QuantideX NGS RNA Lung Cancer
Qiagen® : RNeasy FFPE Kit	Illumina® TST170
	nCounter® nanoString

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Sample type (FFPE)	Fusion gene transcript (ref lab.)
Cell line	*SLC34A2* exon 4 - *ROS1* exon 23
Cell line	*CCDC6* exon 1- *RET* exon 12
Cell line	*TPM3* exon 8 - *NTRK1* exon 9/11
Lung tissue	*KIF5B* exon 24 - *ALK* exon 20
Lung tissue	*MET* exon14 skipping

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Sample type (FFPE)	Fusion gene transcript (ref lab.)	Same result	No result
Cell line	*SLC34A2* exon 4 - *ROS1* exon 23	6 / 6	
Cell line	*CCDC6* exon 1- *RET* exon 12	6 / 6	
Cell line	*TPM3* exon 8 - *NTRK1* exon 9/11	6 / 6	
Lung tissue	*KIF5B* exon 24 - *ALK* exon 20	5 / 6	1 / 6
Lung tissue	*MET* exon14 skipping	2*/ 6	4# / 6

* With remark of poor RNA quality
\# insufficient RNA quality

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- CCDC6-RET fusie
- CCDC6-RET fusietranscript
- CCDC6-RET translocatie (exon 1- exon 12)
- RET fusietranscript positief: CCDC6_1-RET_12
- RET fusie, RET NM_020630 exon12 : CCDC6 NM_005436.4 exon1
- CCDC6 : RET fusie: CCDC6: NM_005436.4:exon:1 ; RET: NM_020630.4:exon:12
- CCDC6-RET, CCDC6{ENST00000263102}:r.1_535_RET{ENST00000355710}:r.2369_565

PATH fusion gene analysis - round robin

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KMBP overleg 4 okt 2019

Microscopie:

CCDC6-RET fusietranscript
CCDC6 [NM_005436.4] exon 1; RET [NM_020630.4] exon12

Moleculaire interpretatie:

CCDC6-RET fusietranscript positief

- CCDC6-R
- CCDC6-R
- CCDC6-R
- RET fusi
- RET fusi
- CCDC6 -
- CCDC6-RET, CCDC6{ENST00000263102}:r.1_535_RET{ENST00000355710}:r.2369_565

Conclusions

- Gene fusion events are infrequent in NSCLC, but are actionable
- Gene fusion events are not limited to NSCLC
- Number of actionable fusions proteins is increasing
- Biological and clinical relevance of the different fusion partners is unclear
- Gene fusion transcripts can be reproducibly identified with different technologies
- Consensus on description gene fusion transcripts (→ PALGA protocol module)

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