Возможности и перспективы ПЦР-диагностики транслокаций ALK и ROS1

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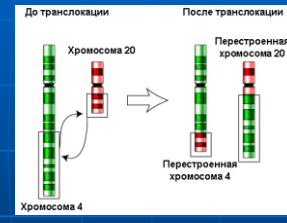
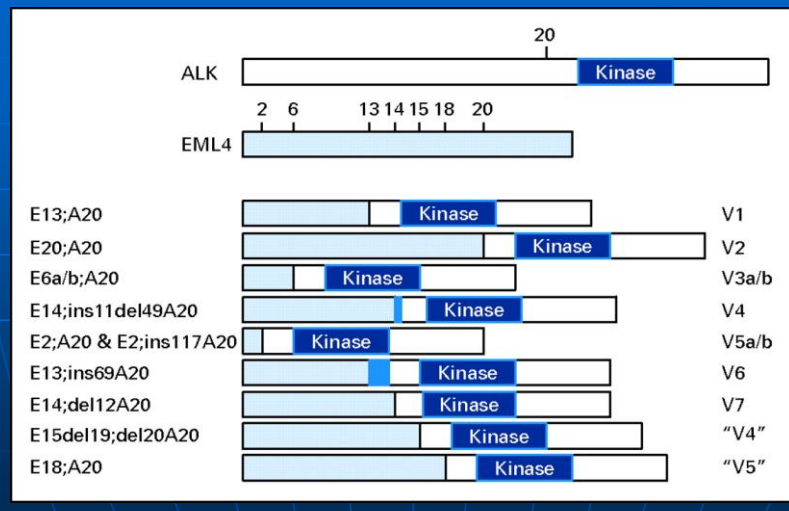
Термины

- Транслокация (translocation) – хромосомная перестройка, при которой 2 хромосомы (2 гена) обмениваются своими фрагментами



- Синонимы: химеры (chimeras), «слитные гены» (gene fusions), перестройки (rearrangements)
- Филадельфийская хромосома (хронический миелолейкоз): t (9;22) BCR/ABL

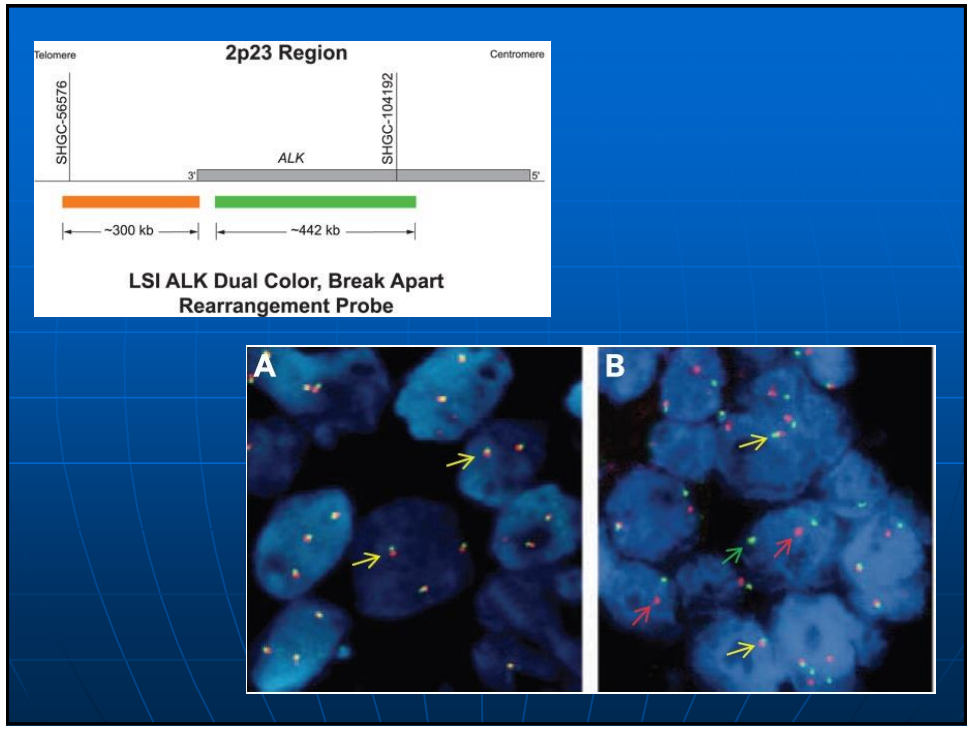
Транслокации EML4-ALK

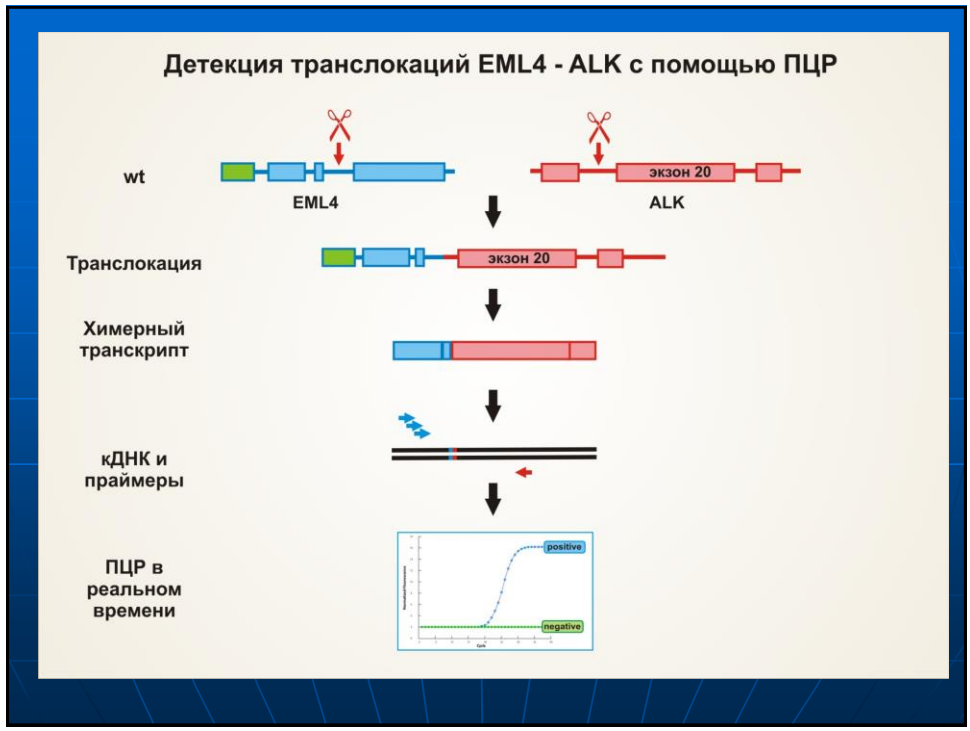


Horn and Pao, 2009

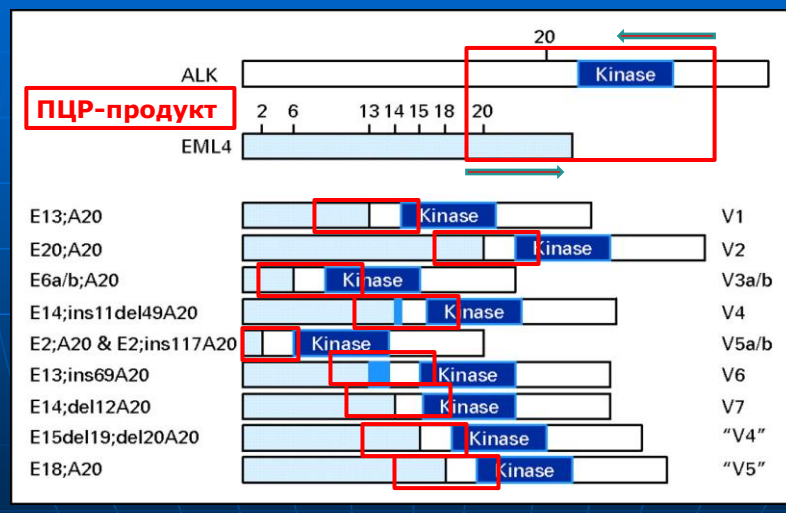
Транслокации EML4/…-ALK

- Разные варианты транслокаций
- Разные гены-партнёры
- Технические трудности диагностики


Telomere
2p23 Region
Centromere
SHGC-56576
SHGC-104192
ALK
3'
5'
~300 kb
~442 kb
LSI ALK Dual Color, Break Apart Rearrangement Probe
A
B

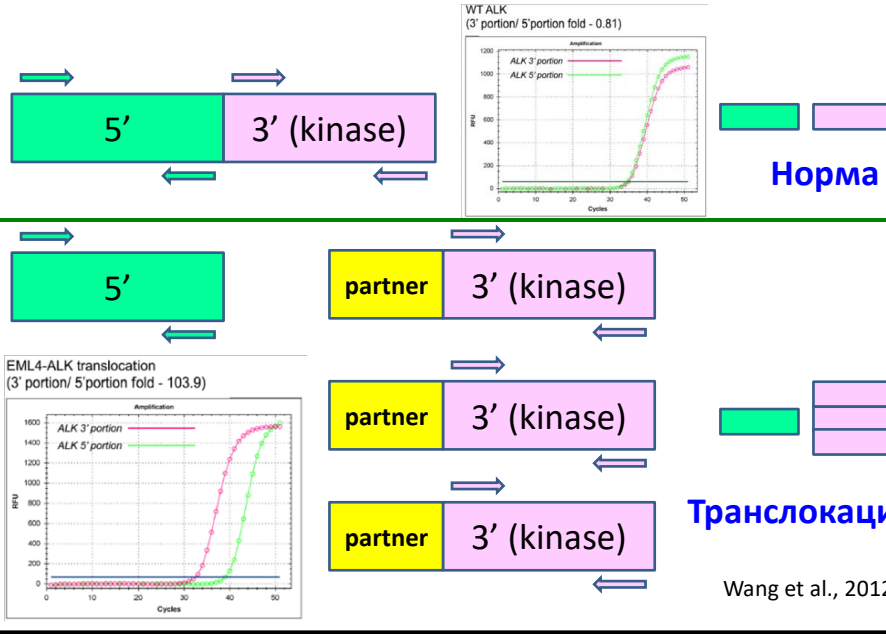

Детекция транслокаций EML4 - ALK с помощью ПЦР
wt
EML4
экзон 20
ALK
Транслокация
экзон 20
Химерный транскрипт
кДНК и праймеры
ПЦР в реальном времени
positive
negative

ПЦР-детекция транслокаций EML4-ALK



Horn and Pao, 2009

«Универсальный» ПЦР-тест: детекция несбалансированной экспрессии 5'/3'-концевых последовательностей ALK



Wang et al., 2012

Contents lists available at ScienceDirect
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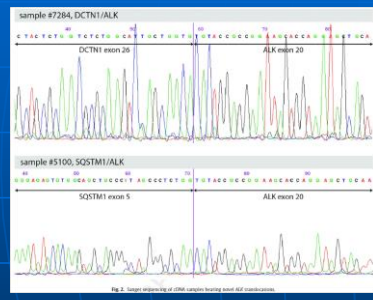
journal homepage: www.elsevier.com/locate/canlet

Original Articles

Novel *ALK* fusion partners in lung cancer

Aglaya G. Iyevleva [a,b], Grigory A. Raskin [c], Vladislav I. Tiurin [a], Anna P. Sokolenko [a,b], Natalia V. Mitiushkina [a], Svetlana N. Aleksakhina [a], Aigul R. Garifullina [a], Tatiana N. Strelkova [a], Valery O. Merkulov [a], Alexandr O. Ivantsov [a], Ekatherina Sh. Kuligina [a], Kazimir M. Pozharisski [a,b], Alexandr V. Togo [a], Evgeny N. Imyanitov [a,b,d,*]



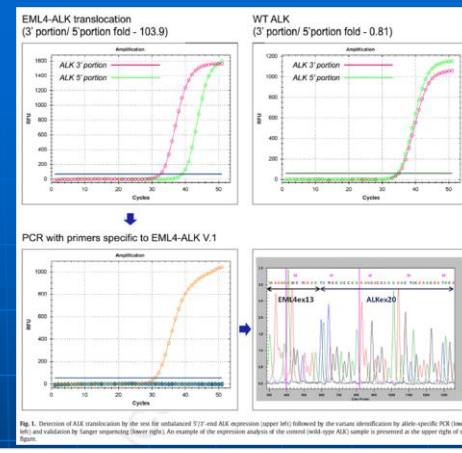


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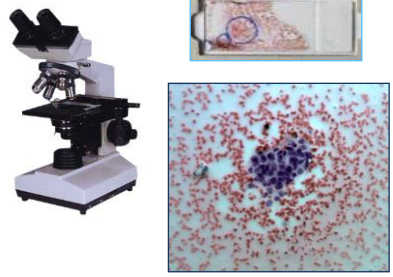
Table 2
Comparison of break-apart FISH and PCR assays.

Sample groups	FISH+	FISH–
Unbalanced ALK+ and variant-specific RT-PCR+ (n = 6)	6 (100%)	0 (0%)
Unbalanced ALK+ and variant-specific RT-PCR– (n = 5)	2* (40%)	3 (60%)
Unbalanced ALK– and variant-specific RT-PCR+ (n = 1)	1 (100%)	0 (0%)
Unbalanced ALK– and variant-specific RT-PCR– (n = 43)	0 (0%)	43 (100%)

* NGS analysis revealed novel translocations in both these samples.

Detection of *EGFR* Mutations and *EML4-ALK* Rearrangements in Lung Adenocarcinomas Using Archived Cytological Slides

Natalia V. Mitiushkina, PhD[1]; Aglaya G. Iyevleva, MD, PhD[1,2]; Artiom N. Poltoratskiy, MD[3]; Alexandr O. Ivantsov, MD, PhD[4]; Alexandr V. Togo, PhD[1]; Igor S. Polyakov, MD, PhD[5]; Sergey V. Orlov, MD, PhD[3]; Dmitry E. Matsko, MD, PhD[4]; Viktor I. Novik, MD, PhD[6]; and Evgeny N. Imyanitov, MD, PhD[2,7,8]



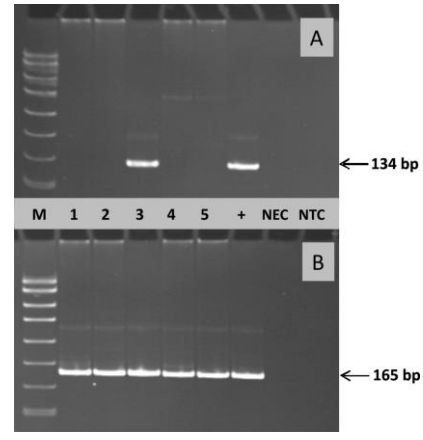


TABLE 1. Success Rate for DNA and RNA Isolation From Cytological and Histological Lung Cancer Samples[a]

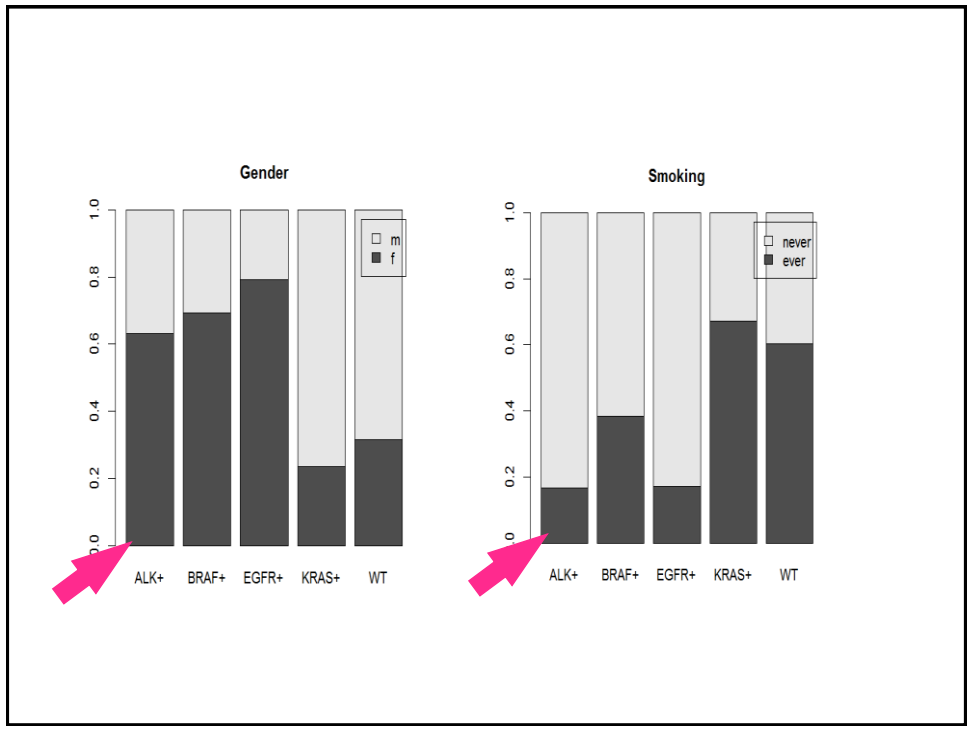
DNA/RNA	Cytological Samples	Histological Samples	No of Informative Sample Pairs
DNA isolation (n=75)	73 (97%)	74 (99%)	72 (96%)
RNA isolation (n=44)	42 (95%)	38 (86%)	36 (82%)

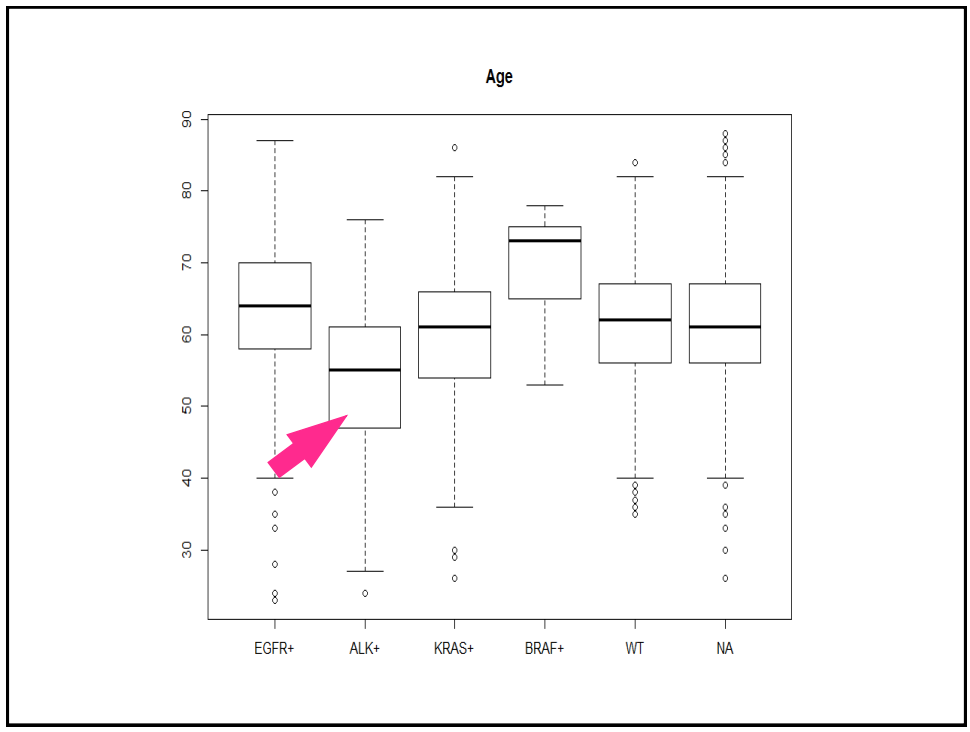
TABLE 3. *EGFR* and *EML4-ALK* Testing in Cytological and Histological Lung Cancer Samples

Test	*EGFR* Mutations (n=72)	*EML4-ALK* Translocations (n=36)
Concordant pairs		
Wild-type/wild-type	54 (75%)	32 (89%)
Mutation/mutation	14 (19%)	4 (11%)
Total	68/72 (94%)	36 (100%)
Discordant pairs		
Cytology: mutation/ histology: wild-type	3 (4%)	0
Cytology: wild-type/ histology: mutation	1 (1%)	0
Total	4 (6%)	0

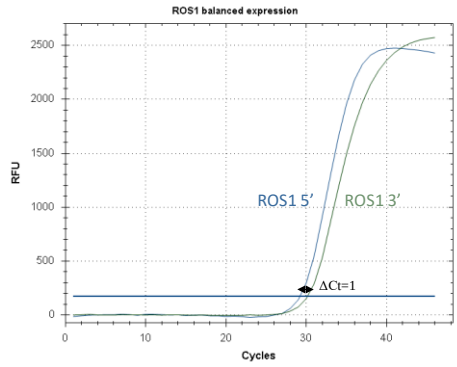
Тестирование мутаций

	Курившие	Не курившие	Статус курения не известен	Все больные	Сравнение частот у куривших и не куривших, тест Хи-квадрат
EGFR-мутации (L858R и ex19del)	42/584 (7%)	204/572 (36%)	224/1180 (19%)	470/2336 (20%)	p < 2.2e-16
Транслокации ALK	6/542 (1%)	30/368 (8%)	59/956 (6%)	95/1866 (5%)	p = 2.247e-07
Мутации KRAS	143/463 (31%)	70/298 (23%)	181/609 (30%)	394/1370 (29%)	p = 0.03646
Мутации BRAF	5/278 (1,8%)	8/183 (4%)	-	13/461 (2,8%)	p = 0.1947

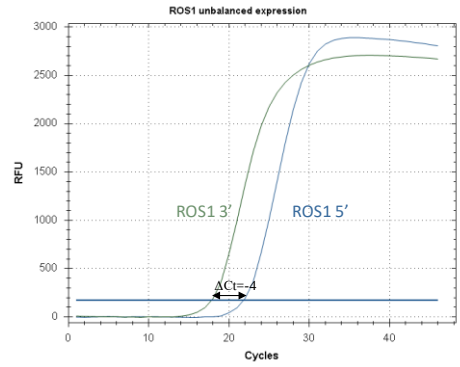

Gender
m
f
0.0
0.2
0.4
0.6
0.8
1.0
ALK+
BRAF+
EGFR+
KRAS+
WT
Smoking
never
ever
0
0.2
0.4
0.6
0.8
1.0
ALK+
BRAF+
EGFR+
KRAS+
WT


Age
30
40
50
60
70
80
90
EGFR+
ALK+
KRAS+
BRAF+
WT
NA

Test for ROS1 5’/3’ unbalanced expression

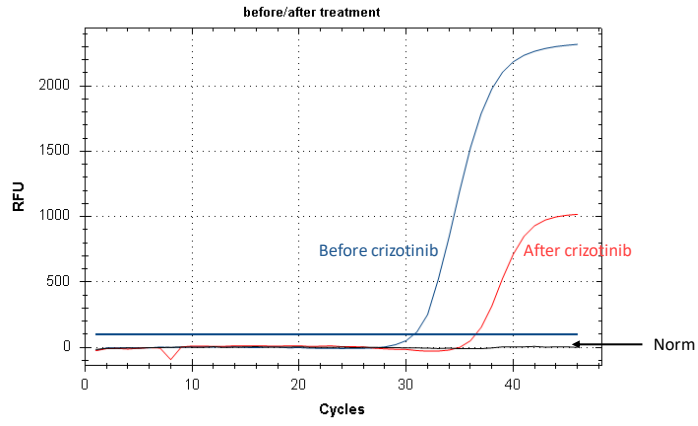


Balanced expression
(norm)



Unbalanced expression
(ROS1 translocation)

ROS1 CD74ex6/ROS1ex34 rearrangement before and after crizotinib



NO complete molecular response

ROS: собственные данные

- Частота: 7/599 (согласуется с данными литературы)
- Некурящие