



Московская Городская
Онкологическая Больница № 62
ДЕПАРТАМЕНТ ЗДРАВООХРАНЕНИЯ Г. МОСКВЫ

Поиск редких генетических аномалий – потенциальных мишеней для таргетной терапии у пациентов с НМРЛ

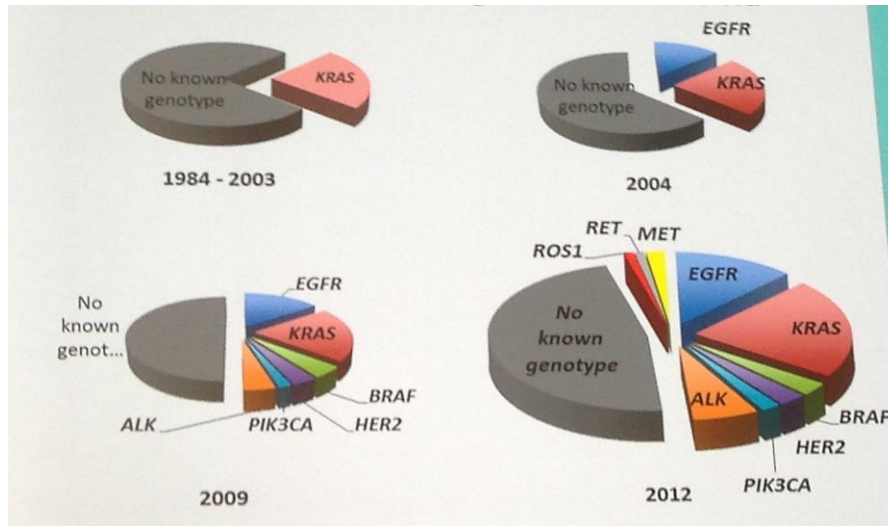
Гикало М.Б., Баринов А.А., Гагарин И.М., Демидова И.А.

Москва 07.04.2017

Немелкоклеточный рак лёгкого (НМРЛ) – от исследований к ежедневной практике

- Объект пристального внимания исследователей с 2004г.
- Одна из наиболее полно охарактеризованных онкопатологий в проекте TCGA (*The Cancer Genome Atlas*)
- Исследование минимум 3 генетических маркеров для определения возможности назначения таргетной терапии (*EGFR, ALK, ROS1*)

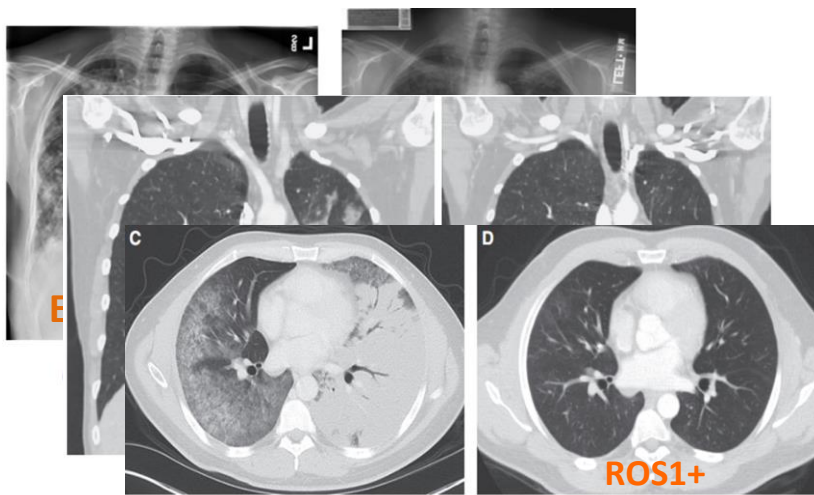
Открытие роли *EGFR* – переломный момент в исследовании НМРЛ



Meyerson M, IASLC 2013

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Клиническая эффективность таргетной терапии



Pao&Miller, 2004; Kwak et al, NEJM 2010; Bergethon K, JCO 2012



ARTICLE

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Comprehensive molecular profiling of lung adenocarcinoma

The Cancer Genome Atlas Research Network*

Adenocarcinoma of the lung is the leading cause of cancer death worldwide. Here we report molecular profiling of 250 resected lung adenocarcinomas using messenger RNA, microRNA and DNA sequencing, integrated with copy number, methylations and protein analysis. High rates of somatic mutation were seen (mean 8.9 mutations per megabase). Eighteen genes were statistically significantly mutated, including EGF activating mutations and novel driver genes. A mutation which is recurrently enriched with local MYC amplification, EGFR mutations were more frequent in female patients, whereas mutations in ERBB2 were more common in males. Alterations in NF1, MET, ERBB2 and RET occurred in 13% of cases and were enriched in samples otherwise lacking an activated oncogene, suggesting a driver role for these genes in certain tumours. DNA and miRNA sequences from the same tumour highlighted striking alterations driven by somatic genomic changes, including those in EMT, miR-10b-1, miR-10b-2, miR-10b-3, miR-10b-4 and EMT16, indicating activity when measured at the protein level, was explained by known mutations in only a fraction of cases, suggesting additional, unexplained mechanisms of pathway activation. These data establish a foundation for classification and further investigations of lung adenocarcinoma molecular pathogenesis.

Lung cancer is the most common cause of global cancer-related mortality, leading to over 1 million deaths each year and adenocarcinoma is the most common histological type. Smoking is the major cause of lung adenocarcinoma but, as smoking rates decline, proportionally more cases occur in never-smokers (defined as those in the algorithm in this study). Recently, molecularly targeted therapies have dramatically improved treatment for patients whose tumours harbour somatically activated oncogenes such as mutant EGFR or rearranged ALK. EGFR is in *ROS1* (6.2–14.1), *MET* (2.6) and *ERBB2* (2.1) are also oncogenes targeted by these therapies. However, most lung adenocarcinomas do not have identifiable driver oncogenes and further mutations in EGFR and other kinases will result with conventional chemotherapy. Tumour suppressor gene alterations, such as those in *TP53*, *SMAD4*, *CCNE1*, *CCNE2*, *CCNE3*, *CCNE4*, *CCNE5*, and *CCNE6*, are the most common in lung adenocarcinoma and are generally thought to be inactivated. Finally, lung adenocarcinoma shows high rates of somatic mutations and protein overexpression, challenging classification of it but the most frequent driver gene alteration because of large burden of passenger oncogene tumour genes^{1–11}. Our efforts focused on comprehensive, multiplatform analysis of lung adenocarcinoma, with extensive research profiling and clinically actionable events.

Clinical samples and histopathologic data

We analysed tumour and matched normal material from 250 previously untreated lung adenocarcinoma patients in the period between 2007 and 2012 (Supplementary Table 1). All major histologic types of lung adenocarcinoma were represented: 1% papillary, 33% acinar, 49% papillary, 14% micropapillary, 2% solid, 4% sarcomatous, 6% tubular and 4% undifferentiated adenocarcinoma (Supplementary Fig. 1). Median follow-up was 19 months, and 163 patients were alive at the time of last follow-up. Eighty-one percent of patients responded to first-generation EGFR tyrosine kinase inhibitors (TKIs), 20% to second-generation TKIs and 10% to crizotinib. Tumour and normal tissue were genotyped at 50,000 loci across the genome using Illumina Infinium arrays. Somatic mutations were identified using the Mutect2 algorithm and are catalogued in the somatic mutation database (SMDB). The somatic mutation database (SMDB) contains somatic mutations identified in tumour samples and is available at <http://cancer.sanger.ac.uk/cancer-genome>. Somatic mutations were identified using the MuTect2 algorithm and are catalogued in the somatic mutation database (SMDB). The somatic mutation database (SMDB) contains somatic mutations identified in tumour samples and is available at <http://cancer.sanger.ac.uk/cancer-genome>. Somatic mutations were identified using the MuTect2 algorithm and are catalogued in the somatic mutation database (SMDB). The somatic mutation database (SMDB) contains somatic mutations identified in tumour samples and is available at <http://cancer.sanger.ac.uk/cancer-genome>.

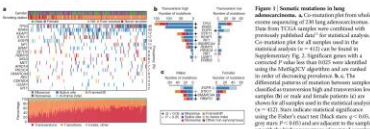
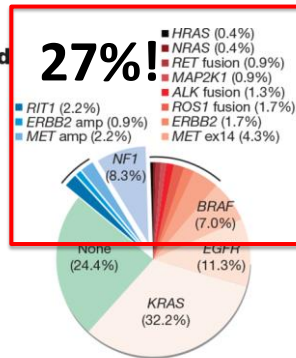


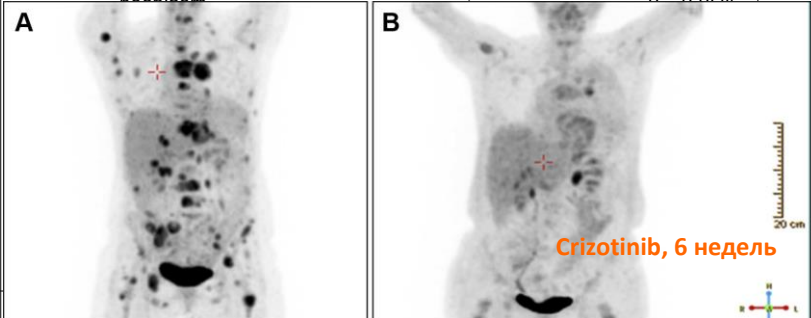
Figure 1 Somatic mutations in lung adenocarcinoma. A heatmap of somatic mutations across the genome for 250 lung adenocarcinoma samples. The x-axis represents chromosomes 1-22, X, and Y. The y-axis represents the number of mutations per megabase (mut/Mb). A color scale indicates the density of mutations, with red representing high density and blue representing low density. A vertical line indicates the location of the MET gene on chromosome 7.

Аденокарцинома лёгкого в проекте TCGA (The Cancer Genome Atlas, 2014)



Частота встречаемости мутаций в 14-м экзоне MET

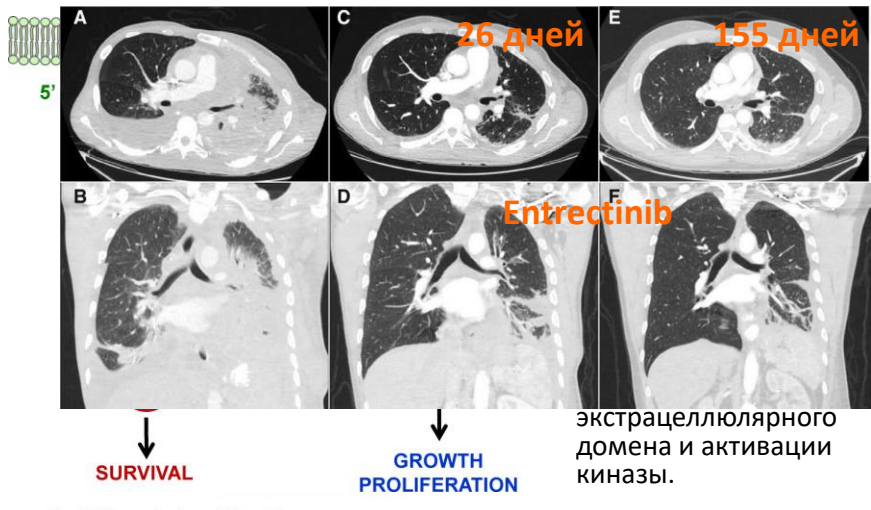
Заболевание	MET exon 14	Всего	Частота	Lower - Upper 95% CI
Аденокарцинома лёгкого	131	4,402	3.0%	2.5 – 3.5%
Другие новообразования в лёгком	62	2,669	2.3%	1.8 – 3.0%
Brain glioma	6	1,708	0.4%	0.1 – 0.8%
Tumors of unknown primary origin	15	3,376	0.4%	0.2 – 0.7%
Female reproductive system	0	7,436	0%	0 – 0.05%



Frampton GM et al. WLCC 2015



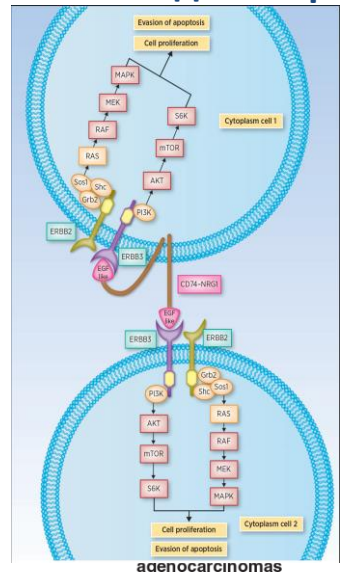
Перестройки NTRK1



Doebele et al. Cancer Discovery 2015



NRG1. Инвазивная муцинозная аденокарцинома лёгкого



Stage	Smoking status	AD subtype
1b	Never	Invasive mucinous
1a	Never	Invasive mucinous
1a	Never	Invasive mucinous
1a	Never	Invasive mucinous

RET negative

- NRG1, 2, 3, 4 = факторы роста, запускающие активацию ERBB2-ERBB3 рецепторов.
- CD74-NRG1, SCL3A2-NRG1 способствуют неконтролируемой пролиферации клеток за счёт активации внутриклеточных сигнальных путей.

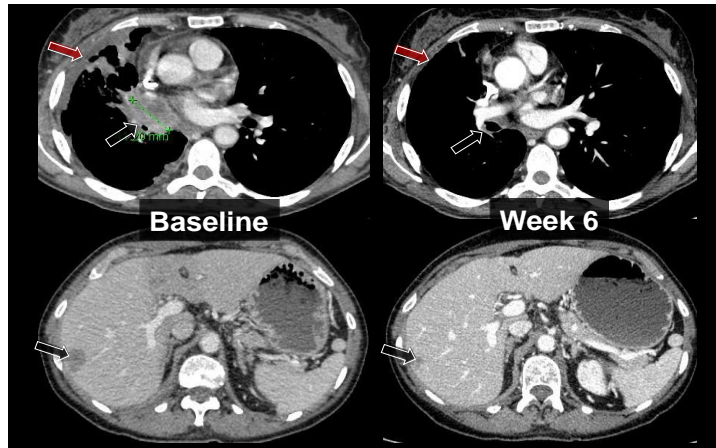
Fernandez-Cuesta & Thomas, 2015



BRAF при НМРЛ

Carter et al / BRAF MUTATION DETECTION BY NGS

BRAFmut patient treated w Dabrafenib (trial,

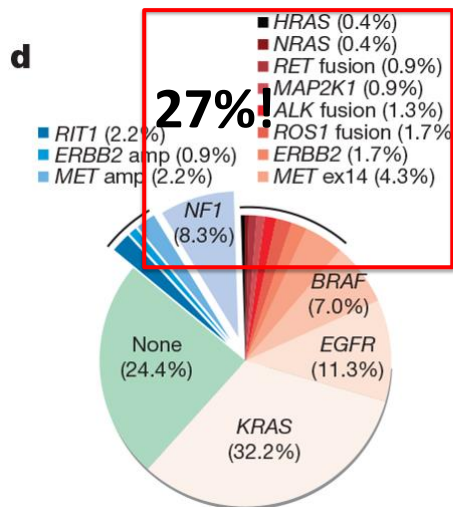


Carter et al, AJCR 2015; Courtesy of Roman Thomas and Fabrice Barlesi PLC 2014

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Аденокарцинома лёгкого (TCGA , 2014)



TCGA 2014

Как отыскать иголку в стоге сена быстро и недорого ?



- **Как минимизировать время и затраты на молекулярную диагностику с максимальной пользой для пациента?**
- ПЦР, классическое секвенирование, ИГХ, FISH – недели и десятки тысяч рублей
- Полногеномное секвенирование – RUO, за пределами дорого
- Коммерческие панели – не ориентированы на реальные потребности пользователей
- **Существует острая необходимость разработки custom-панелей для NGS с учётом новых генетических маркеров**

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Собственные исследования



- **I этап** – поиск редких генетических нарушений в группе пациентов с диагнозом аденокарцинома лёгкого с использованием рутинных методов.
- **II этап** – создание NGS-панели для таргетного ресеквенирования кДНК

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Алгоритм исследования

- 200 пациентов с диагнозом аденокарцинома лёгкого
- EGFR- и ALK-негативные
- FFPE и операционный материал

I этап

• KRAS ex 2
• BRAF ex 11, 15
ПЦР, Sanger

• HER2 ex 20
• MET ex 14
FLA

• ROS1, RET,
MET, NTRK1,
NRG1, BRAF
FISH

II этап

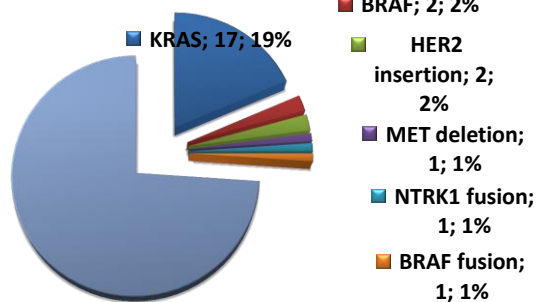
Создание панели
для таргетного
ресеквенирования
кДНК

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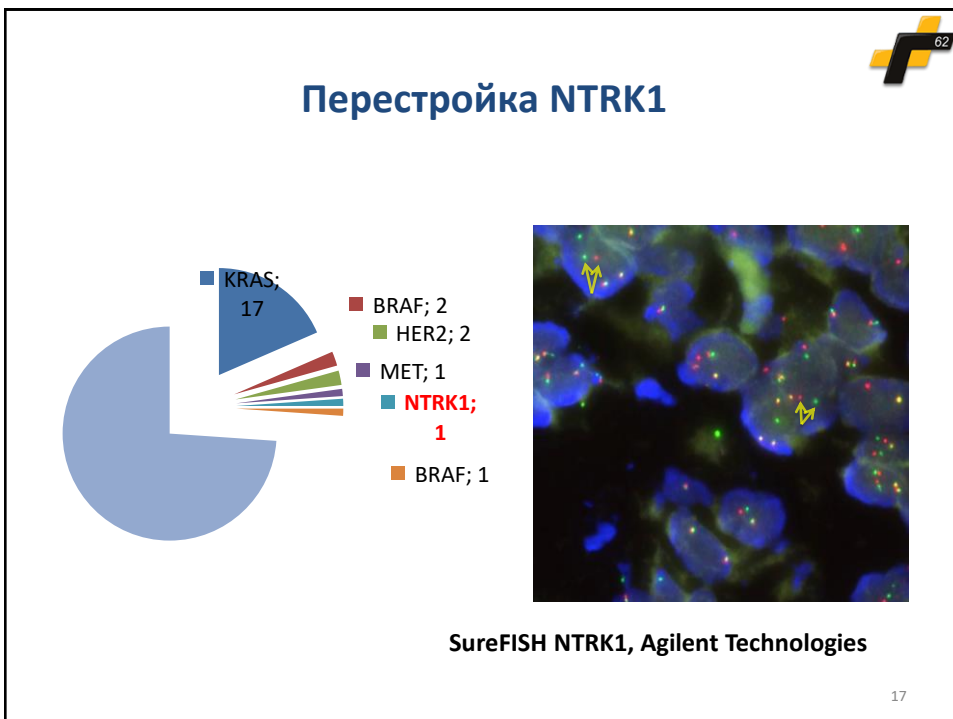
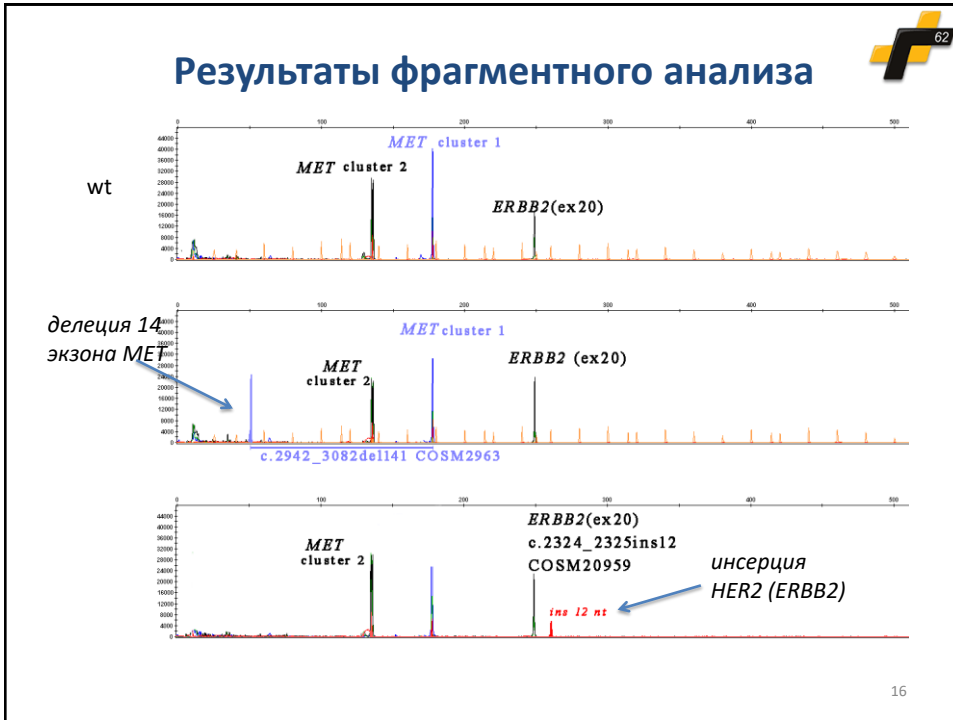


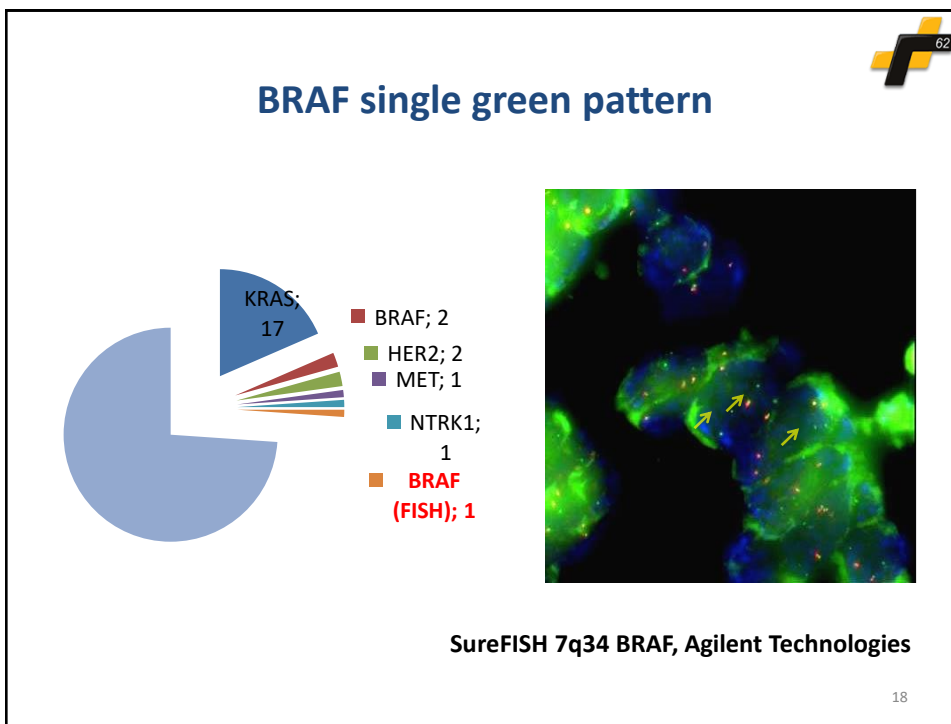
Предварительные результаты (n=92)

		Мужчины (n=62)	Женщины (n=30)
Возраст	<60	18 (29%)	8 (37%)
	≥60	44 (71%)	22 (63%)
Статус курения	Курящий	44 (71%)	4 (13%)
	Некурящий	18 (29%)	26 (87%)



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II этап исследований. NGS-панели для таргетного ресеквенирования кДНК

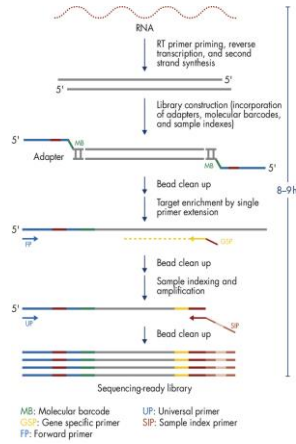
- Состав панели. ALK, ROS1...
- Поиск известных или неизвестных партнёров транслокации?
- Возможность работать с РНК, выделенной из парафиновых блоков.
- Экономическая эффективность

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QIaseq Targeted RNA Panel. Human Lung Cancer Panel

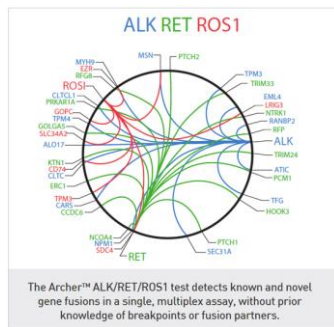


- 23 транслокации
- ALK, ROS1, RET, NTRK1, NRG1

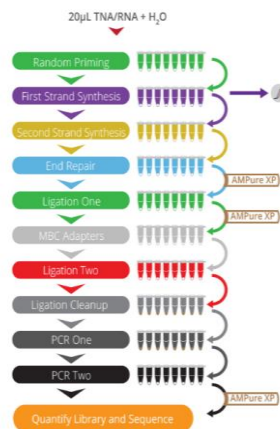


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FusionPlex ALK, RET, ROS1 v2 Kit



Позволяет находить и анализировать неизвестных партнёров транслокации



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Благодарю за внимание!

