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The Role of Liquid Biopsies in Modern Oncology

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New York Cancer and Blood Specialists

Learning objectives:

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-Understand the importance of molecular testing in solid tumors.

-Identify the right patient and the right setting to use liquid biopsies.

-Understand the different platforms used and how to leverage the information provided.

Agenda:

- Definitions
- Types of liquid biopsies
- Indications
- Advantages
- Limitations.

Precision Medicine:

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- Innovative approach to prevent and treat different disease processes taking into account the individuals genetic makeup, lifestyle and environmental factors.

- Precision Oncology: approaching cancer based on molecular alterations, tumor microenvironment factors and immunological variables that predict treatment response and outcomes.

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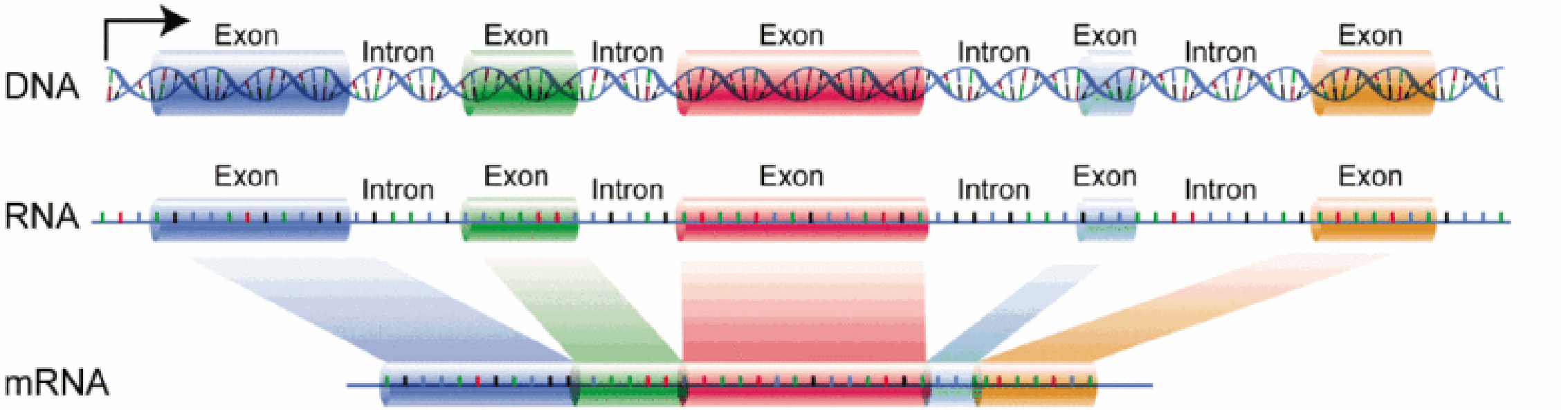

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- **WGS:** costlier than more limited sequencing, equivalent to approximately 3.3 x 10^9 bases (3.3 gigabases [Gb]= 3.3 billion bases).

- **Exome sequencing:** contains the portions of genes that encode proteins 1.5 to 2.0% of the genome (ie, about 30 megabases [Mb]=30 million bases).

National Human Genome Research Institute. (n.d.). Genetics Home Reference. Retrieved October 24, 2023, from https://www.genome.gov/genetics-glossary

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- **Targeted gene panels** – Gene panels provide sequence data for a limited subset of genes (typically 10 to 200 genes). Targeted gene panels are used in settings in which it would be appropriate to sequence many genes to make a diagnosis.

National Human Genome Research Institute. (n.d.). Genetics Home Reference. Retrieved October 24, 2023, from https://www.genome.gov/genetics-glossary

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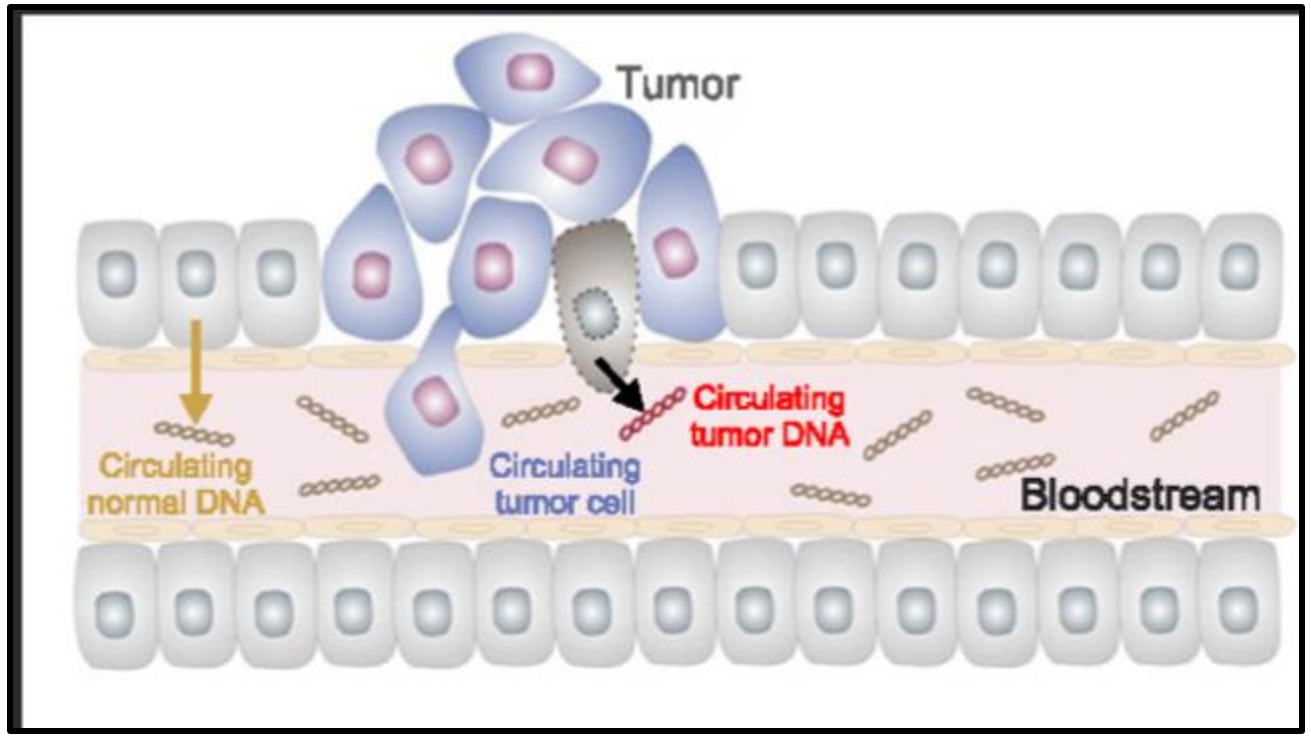
Circulating tumor DNA / Cell Free DNA



Cell free DNA is a mixture of DNA from:

-Blood cells. (90%)

-Viruses

-Solid organs

-Tumors (ctDNA)

ctDNA:

Advantages:

- Detects targetable mutations with faster turnaround time.
- Increases sensitivity when measuring MRD. (almost like a tumor marker).
- Helps detecting resistant mutations.
- Increases the detection of targetable mutations.
- May help with strategies to de-escalate treatment or escalate (being investigated).

Jean G. Bustamante Alvarez, et al, Treatment of Non–Small-Cell Lung Cancer Based on Circulating Cell-Free DNA and Impact of Variation Allele Frequency, Clinical Lung Cancer, Volume 22, Issue 4, 2021

ctDNA

Disadvantages:

- Reimbursement can be an issue in some cases.
- Low tumor burden a low ctDNA shedding can lead to false negatives.
- Misinterpretation of the results depending on the reporting platforms.

Jean G. Bustamante Alvarez, et al, Treatment of Non–Small-Cell Lung Cancer Based on Circulating Cell-Free DNA and Impact of Variation Allele Frequency, Clinical Lung Cancer, Volume 22, Issue 4, 2021

NSCLC

- Objective:
  - Detect targetable mutations.
  - Decrease turn around time and expedite treatment initiation if informative.
  - Detect resistance mutations.

Jean G. Bustamante Alvarez, et al, Treatment of Non–Small-Cell Lung Cancer Based on Circulating Cell-Free DNA and Impact of Variation Allele Frequency, Clinical Lung Cancer, Volume 22, Issue 4, 2021

Utilities/Modalities

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Target Detection

Only blood based

Leighl NB, et al. Clinical Utility of Comprehensive Cell-Free DNA Analysis to Identify Genomic Biomarkers in Patients with Newly Diagnosed Metastatic Non-Small Cell Lung Cancer, Clin Cancer Res 2019

MRD

(tumor marker like)

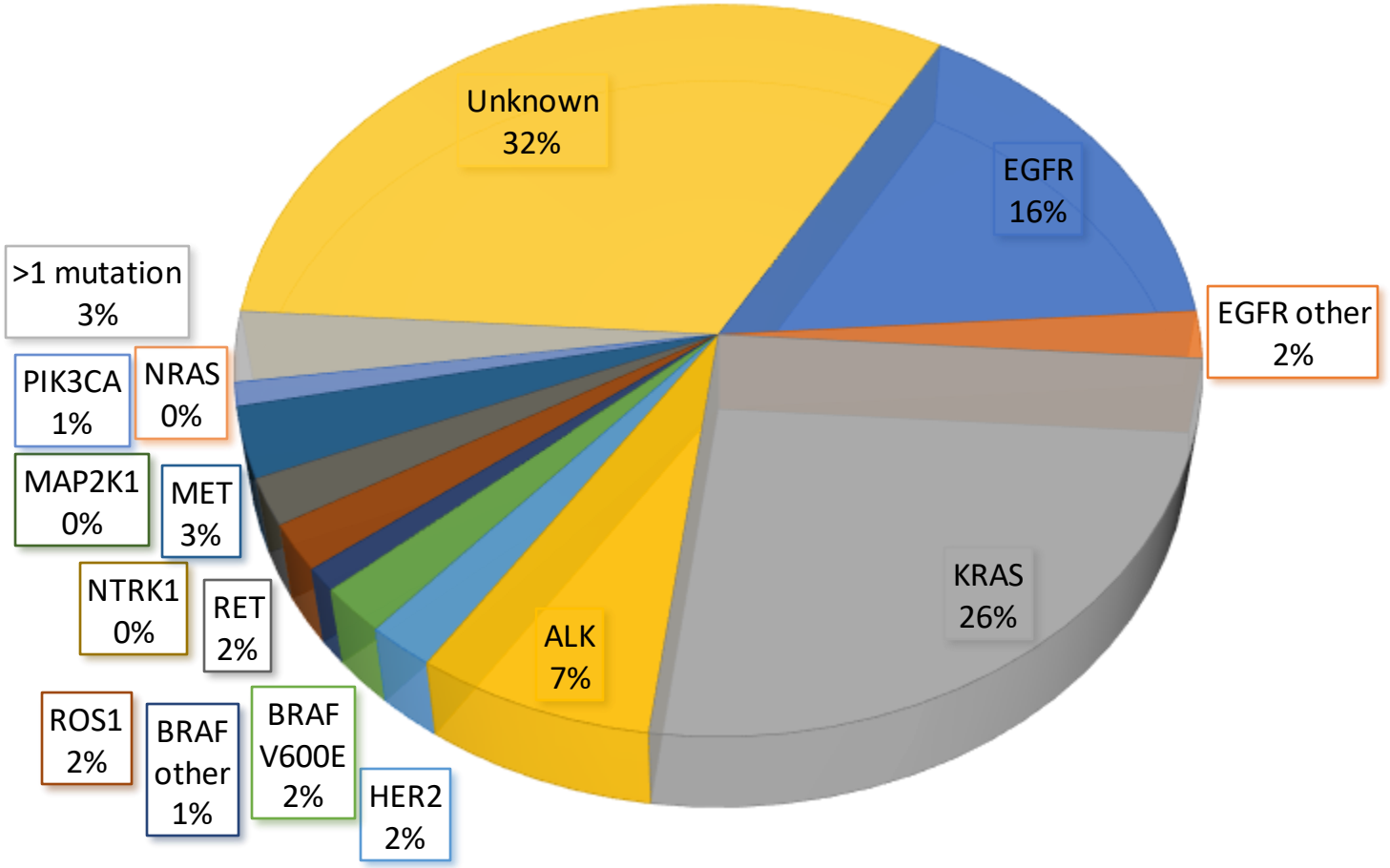
Tumor informed

vs

only blood based

N Engl J Med 2022; 386:2261-2272

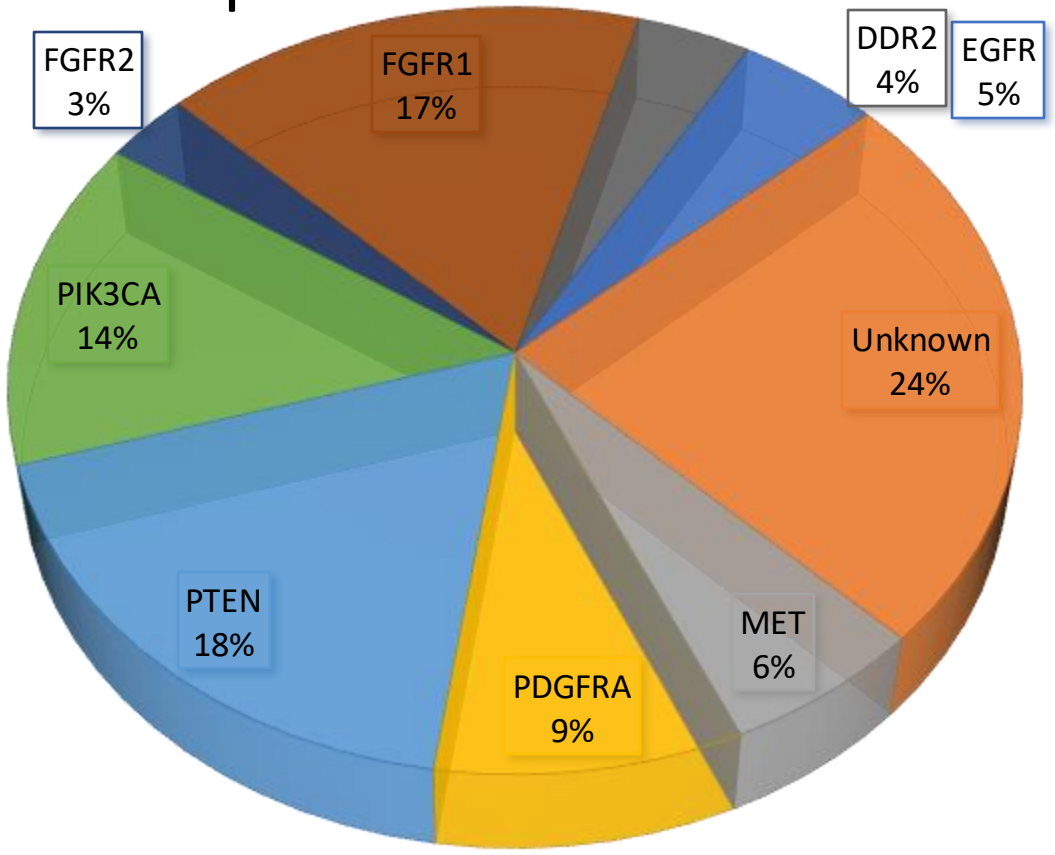
Incidence of driver mutations in lung adenocarcinoma



Mutation	%
EGFR	15%
EGFR other	2%
KRAS	25%
ALK	7%
HER2	2%
BRAF V600E	2%
BRAF other	1%
ROS1	2%
RET	2%
NTRK1	0-5%
MET	3%
MAP2K1	0-5%
PIK3CA	1%
NRAS	0-5%
>1 mutation	3%
Unknown	31%

Lovly C, Horn L, Pao W. 2018 Molecular Profiling of Lung Cancer. My Cancer Genome website. https://www.mycancergenome.org/content/disease/lung-cancer.

Incidence of driver mutations in lung squamous cell carcinoma



Mutation	%
EGFR	5%
Unknown	24%
MET	6%
PDGFRA	9%
PTEN	18%
PIK3CA	14%
FGFR2	3%
FGFR1	17%
DDR2	4%

Lovly C, Horn L, Pao W. 2018 Molecular Profiling of Lung Cancer. My Cancer Genome website. https://www.mycancergenome.org/content/disease/lung-cancer.

Other important nuances:

- Discordant patients could respond to treatment and this phenomena can reflect false negatives in tissue or tumor heterogeneity.
- VAF appears not to predict response.
- Integration of ctDNA/cfDNA demonstrates an increased of the detection of therapeutically targetable mutations.

Jean G. Bustamante Alvarez, et al, Treatment of Non–Small-Cell Lung Cancer Based on Circulating Cell-Free DNA and Impact of Variation Allele Frequency, Clinical Lung Cancer, Volume 22, Issue 4, 2021

Circulating tumor DNA / Tumor informed

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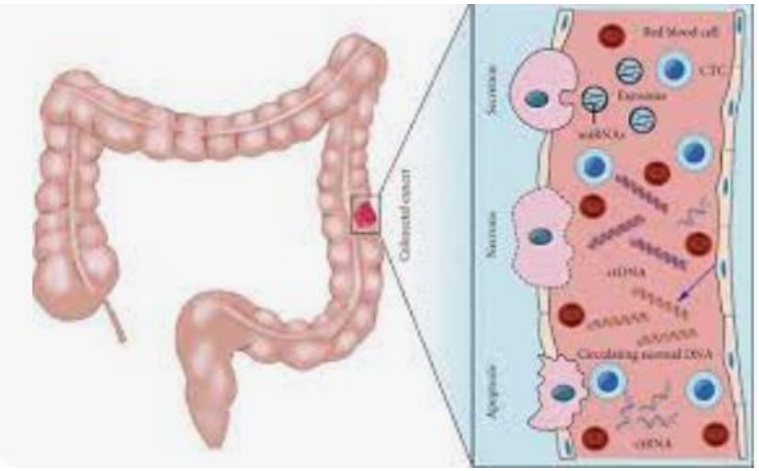


•. 2022 Jun 16;386(24):2261-2272.

The NEW ENGLAND JOURNAL of MEDICINE

RESEARCH SUMMARY

Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer

Tie J et al. DOI: 10.1056/NEJMoa2200075

CLINICAL PROBLEM

The benefit of adjuvant chemotherapy for stage II colon cancer is unclear. Circulating tumor DNA (ctDNA) may provide a biomarker to identify which patients would benefit from adjuvant therapy and which patients might forgo it with minimal risk of recurrence.

CLINICAL TRIAL

Design: A phase 2, randomized, controlled noninferiority trial assessed whether ctDNA-guided management, as compared with standard management, could reduce the use of adjuvant therapy without compromising the risk of recurrence after surgery for stage II colon cancer.

Intervention: 455 patients were randomly assigned in a 2:1 ratio to have treatment decisions guided by either ctDNA results or standard clinicopathological criteria. For ctDNA-guided management, patients with positive ctDNA tests at week 4 or 7 after surgery received fluoropyrimidine or oxaliplatin-based chemotherapy, and those with negative tests at both weeks received no chemotherapy. The primary efficacy end point was recurrence-free survival at 2 years. A key secondary end point was adjuvant chemotherapy use.

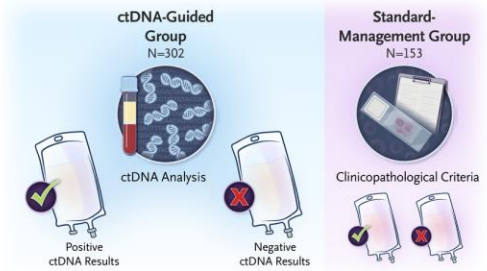
RESULTS

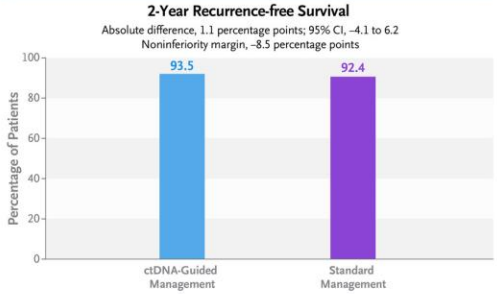
Management guided by ctDNA was noninferior to standard management with respect to 2-year recurrence-free survival and resulted in less use of adjuvant chemotherapy.

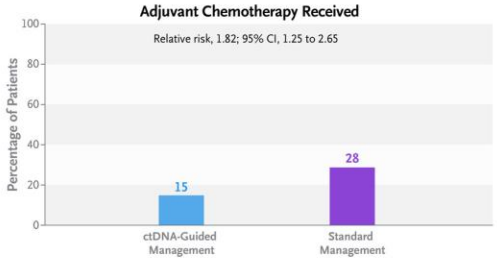
LIMITATIONS AND REMAINING QUESTIONS

- The trial was too small to provide definitive findings for patient subgroups.
- Because management decisions were guided by test results, the patients in the ctDNA-positive and ctDNA-negative subgroups were not randomly assigned to either receive or not receive treatment.
- The effect of a ctDNA-guided strategy was not assessed beyond the initial decision for adjuvant chemotherapy.

Links: Full Article | NEJM Quick Take | Editorial







CONCLUSIONS

Among patients with stage II colon cancer, ctDNA-guided management was noninferior to standard management with respect to 2-year recurrence-free survival and resulted in reduced use of adjuvant chemotherapy.

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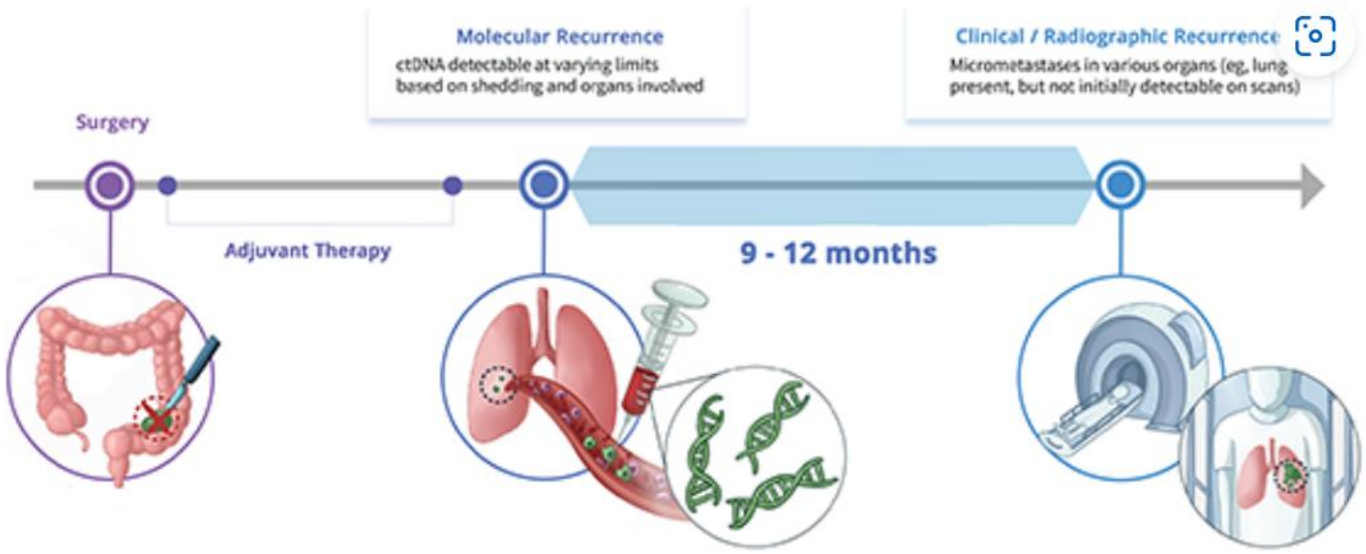
N Engl J Med . 2022 Jun 16;386(24):2261-2272.

Virtually all patients who are ctDNA positive have disease recurrence.



Limitations:

-Negative results

-Positive results after adjuvant chemotherapy.

1.Kasi PM. ctDNA assays: exploring their clinical use in oncology care. ASCO Daily News. January 13, 2022. Accessed November 30, 2022.

Case study:

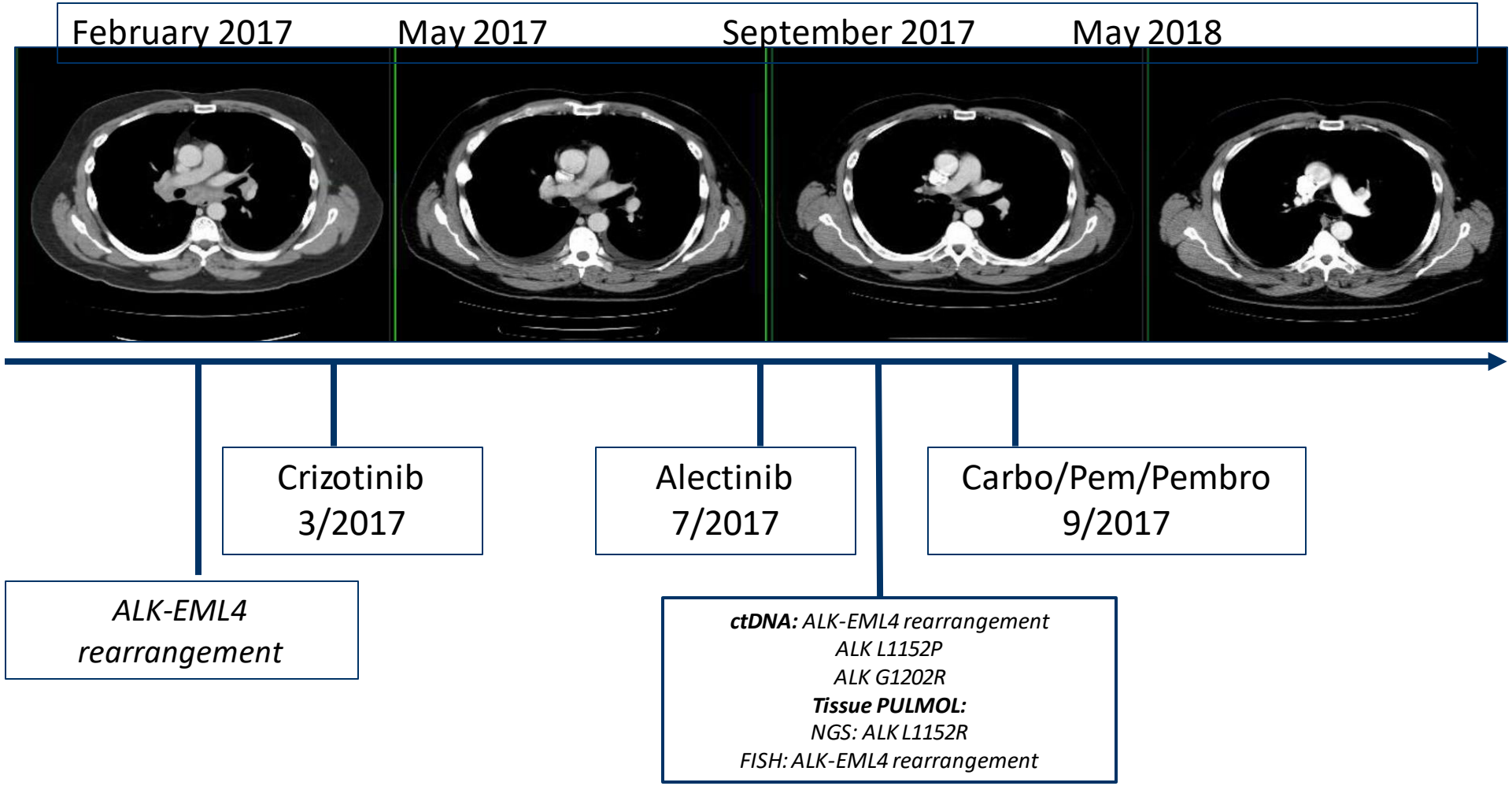
- 2/2017: 49 y.o male, **non-smoker** with FUO, weight loss, pleuritic chest pain.
- CT C/A/P with mediastinal and RP lymphadenopathy and RLL mass.
- Bronchoscopy FNA cytology + for NSCLC (ADC).

What else needs to be requested?

- Brain MRI→Negative.

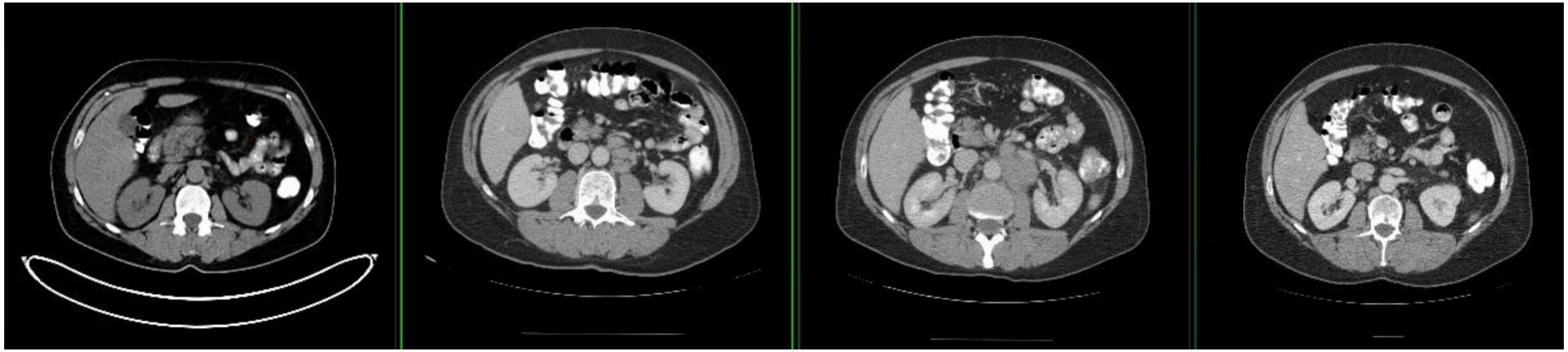
- PD-L1 and molecular studies

Case study:


February 2017
May 2017
September 2017
May 2018
Crizotinib
3/2017
Alectinib
7/2017
Carbo/Pem/Pembro
9/2017
ALK-EML4
rearrangement
ctDNA: ALK-EML4 rearrangement
ALK L1152P
ALK G1202R
Tissue PULMOL:
NGS: ALK L1152R
FISH: ALK-EML4 rearrangement

Case study:

March 2017 July 2017 August 2017 May 2019



Lorlatinib

Crizotinib
3/2017

ctDNA: *ALK-EML rearrangement.*
Tissue PULMOL:
- ALK rearrangement

Alectinib
7/2017

ctDNA: *ALK-EML4 rearrangement*
ALK L1152P
ALK G1202R
Tissue PULMOL:
NGS: ALK L1152R
FISH: ALK-EML4 rearrangement

Carbo + Pemetrexed +
Pembrolizumab 9/2017

ALK resistance mutations

Cellular ALK phosphorylation mean IC_{50} (nmol/L)					
Mutation status	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

Gainor JF, Dardaei L, Yoda S, et al. Molecular Mechanisms of Resistance to First- and Second-Generation ALK Inhibitors in ALK-Rearranged Lung Cancer. *Cancer Discov.* 2016;6(10):1118–1133.

Conclusions:

- ctDNA is here to stay
- “Informative results” are in general actionable or exclude actionable mutations.
- Different platforms have different purposes. (Detect targets, MRD to consider adjuvant, “tumor marker utility”)
- Negative result should be interpreted carefully.
- There are many clinical trials ongoing to assess MRD in different tumors.
- Clinical studies for escalation or De-escalation of adjuvant therapies are ongoing.

Thank you

Questions?

Email: jalvarez@nycancer.com