IX SIMPOSIO · SYMPOSIUM | 2024
BIOPSIA LÍQUIDA · LIQUID BIOPSY
EL CAMINO A LA ONCOLOGÍA DE PRECISIÓN · THE WAY TO PRECISION MEDICINE
25, 26 Y 27 DE ENERO · JANUARY 25th, 26th and 27th

LIQUID BIOPSY APPROACHES FOR THE CLINICAL MANAGEMENT OF LUNG CANCER. RNA-BASED TESTING

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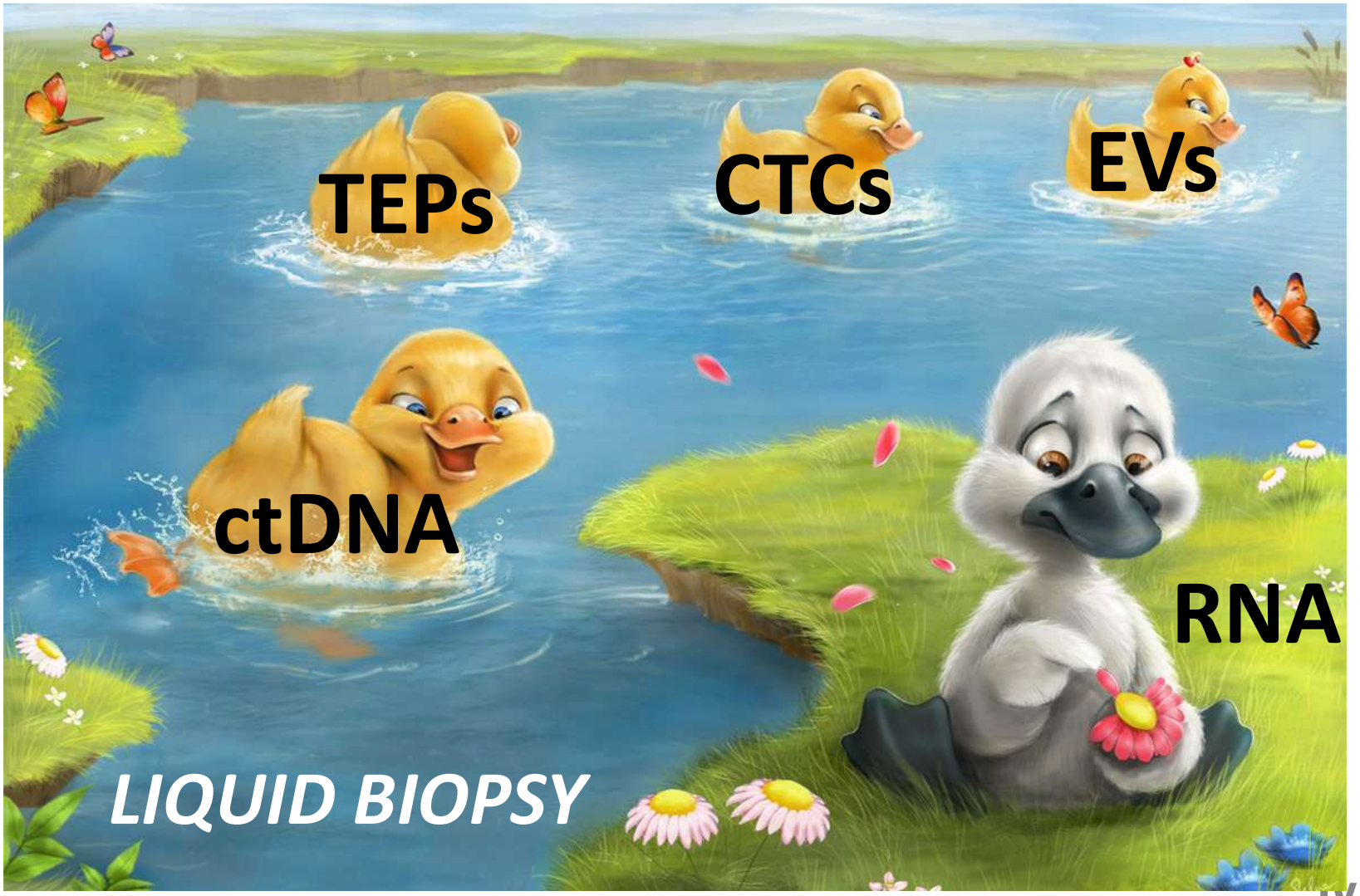
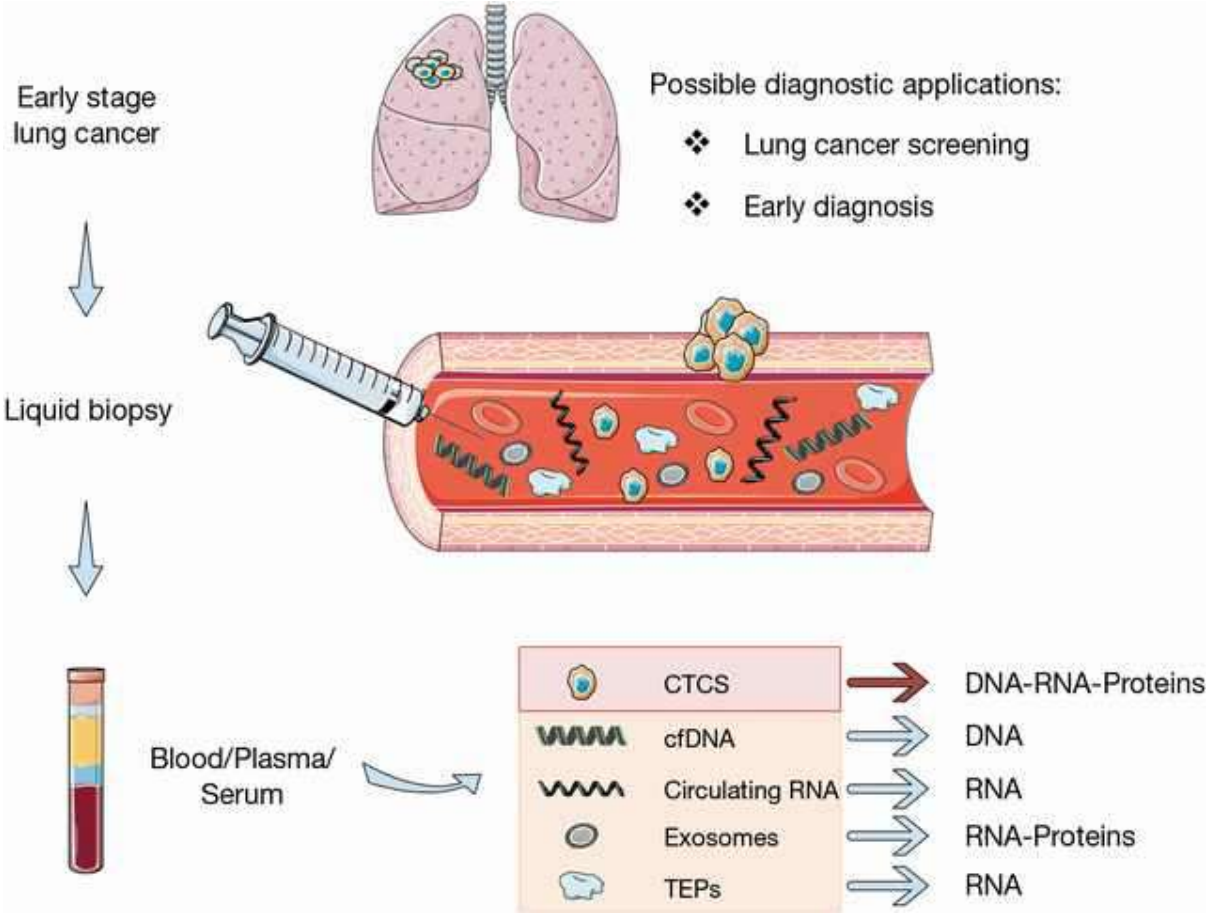

TEPs
CTCs
EVs
ctDNA
RNA
LIQUID BIOPSY

RNA in liquid biopsies



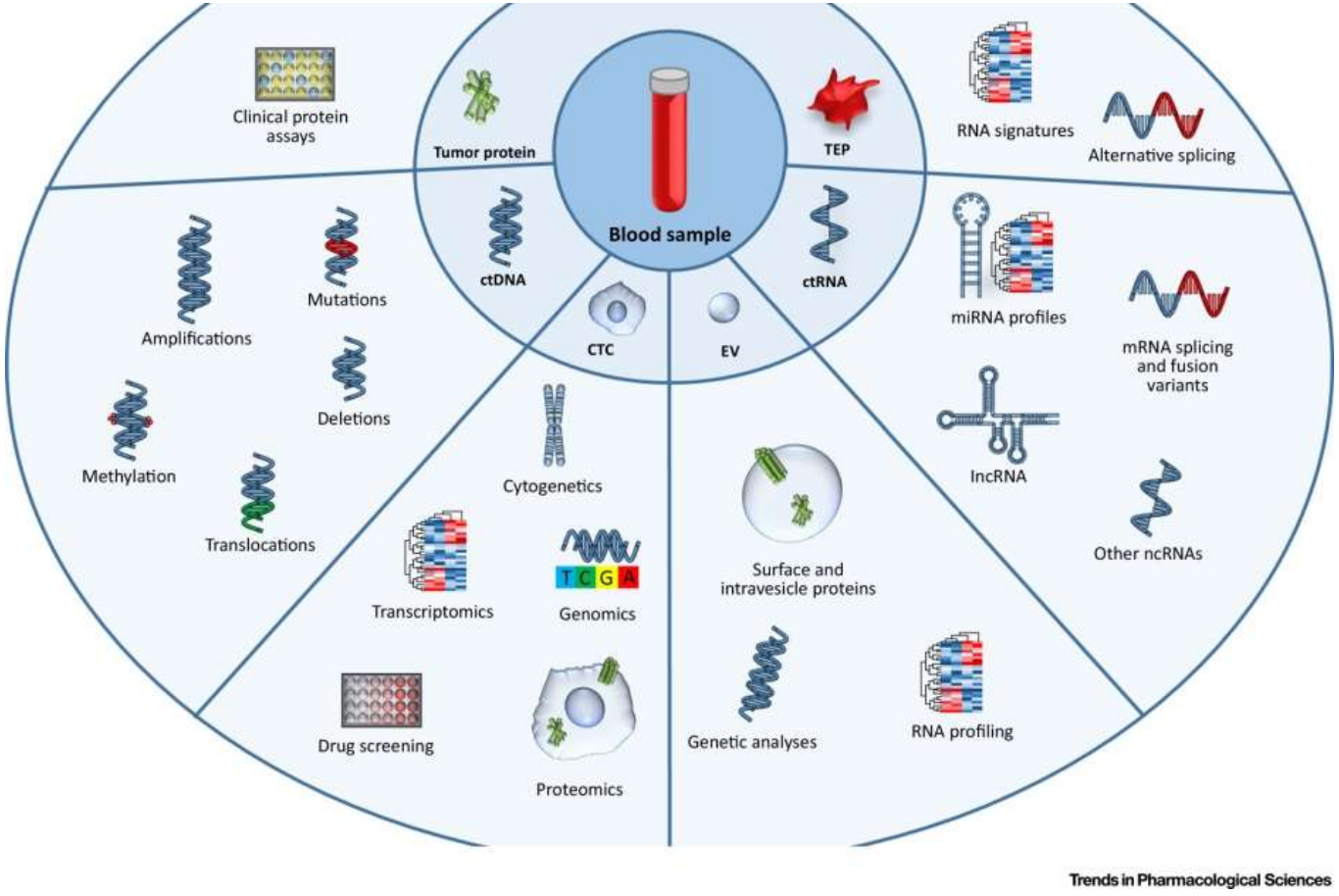
Santarpia et al. JTD, 2018

-Liquid biopsies are revolutionizing cancer testing as a noninvasive method for detection and monitorization of malignancies, complementary or, in some cases, alternative to tumor tissue biopsies.

-Tumor RNA (tRNA) can be isolated from several sources in liquid biopsies

--CTCs
--Exosomes
--Platelets ("tumor educated", TEPs)
--Soluble fraction (ctRNA)

RNA in liquid biopsies (II)



DeRubis et al. TPS, 2019

-Tumor RNA (tRNA) purified from liquid biopsies can be used for two main applications

--Detection of clinically relevant alterations, including splicing (i.e. *METΔ14* or *EGFRvIII*) and fusion variants (*ALK*, *ROS1*, *RET*, *NTRK1-3*, etc)

--Development of gene expression signatures with diagnostic, prognostic and predictive value

-Clinically relevant alterations must be detected on mRNA; while signatures can be based on all types of RNA

Clinically relevant gene fusions and splicing variants in lung cancer

a

Approved biomarkers	ESMO Guidelines (2020)	NCCN Guidelines® (2022)[a]	CAP/IASLC/AMP Guidelines (2018)	ASCO Guidelines (2014)	Pan-Asian Guidelines (2019)
EGFR	●	●	●	●	●
ALK	●	●	●	●	●
ROS1	●	●	●	●	●
BRAF	●	●	●	●	●
NTRK	●	●	●	●	●
PD-L1	●	●	●	●	●

b

Emerging biomarkers	ESMO Guidelines (2020)	NCCN Guidelines® (2022)[a]	CAP/IASLC/AMP Guidelines (2018)	ASCO Guidelines (2014)	Pan-Asian Guidelines (2019)
KRAS[b]	●	●	●	●	●
MET	●	●	●	●	●
RET[b]	●	●	●	●	●
ERBB2/HER2	●	●	●	●	●
TMB[c]	●	●	●	●	●

● Testing recommended ● Expanded panel testing recommended ● Single gene or expanded panel testing recommended ● IHC testing recommended ● No guideline recommendations to date ● Testing not recommended

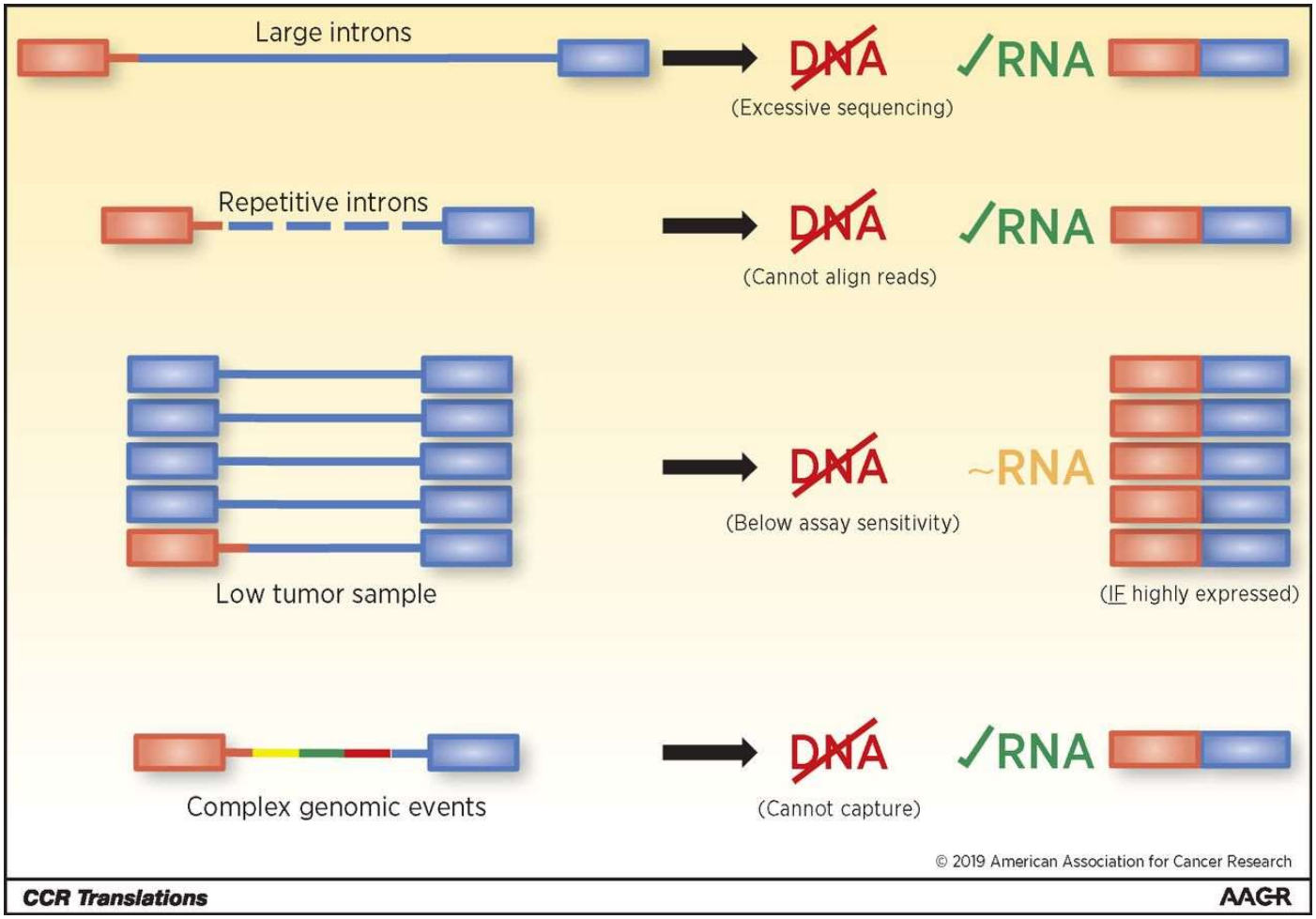
-The ESMO guidelines recommend testing for *ALK*, *ROS1*, *NTRK1* and *RET* fusions, together with *METex14* splicing variant

-Multiplex techniques are needed

-In patients with insufficient or no tissue biopsy available, liquid biopsy is the only alternative

-However, testing for fusions/splicing variants in blood is not incorporated in the routine clinical practice

NGS for detection of gene fusions and splicing variants: DNA vs. RNA



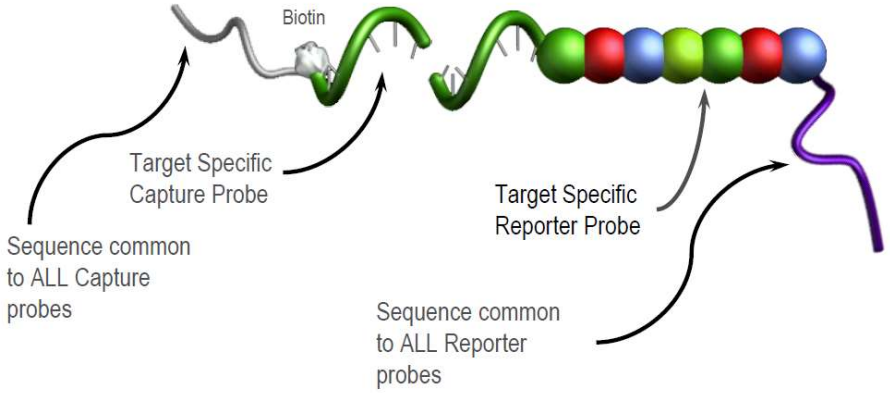
Davies and Aisner, CCR, 2019

-Several studies indicate that multiplex RNA-based techniques are to be preferred over DNA-based NGS for the identification of gene fusions and splicing variants

-In FFPE samples, RNA based panels are widely used for fusion detection (i.e., TruSight™ RNA Fusion Panel, AmpliSeq™ RNA Fusion Lung Cancer Panel, nCounter panels)

-However (suprisingly!!), RNA-based techniques are rarely used to detect fusions and splicing variants in liquid biopsies

nCounter in liquid biopsies: our experience

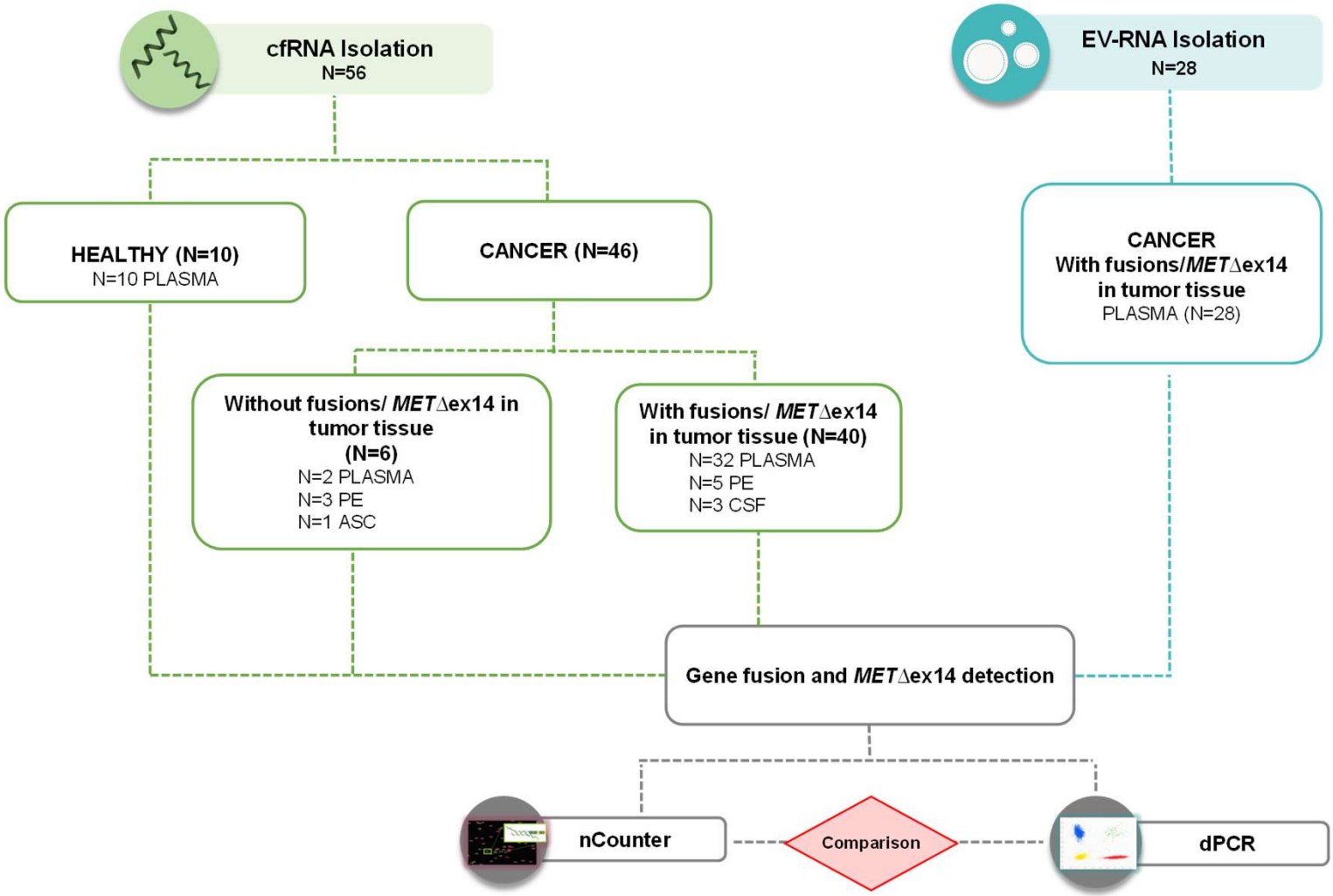


Target
GAPDH
MRPL19
PSMC4
EML4-ALK__E13:A20
EML4-ALK__E18:A20
EML4-ALK__E2:A20
EML4-ALK__E20:A20
EML4-ALK__E6:A20
KIF5B-ALK__K17:A20
TFG-ALK__T5:A20
CCDC6-RET_C1:R12
KIF5B-RET_K15:R11
KIF5B-RET_K16:R12
KIF5B-RET_K22:R12
KIF5B-RET_K23:R12
KIF5B-RET_K24:R11
KIF5B-RET_K24:R8
KIF5B_K15-Common
KIF5B_K24-Common

-29 Specific probes

SDC4-ROS1_S2:R32
SDC4-ROS1_S4:R34
SLC34A2_S4:ROS1-Common
SLC34A2-ROS1_S13del2046:R32
SLC34A2-ROS1_S4:R32
EZR-ROS1_E10:R34
GOPC-ROS1_G4:R36
GOPC-ROS1_G7:R35
TPM3-ROS1_T8:R35
LRIG3-ROS1 _L16:R35
CD74-ROS1_C6:R32
MET_e13_14
MET_e13_15

nCounter in liquid biopsies: our experience



nCounter in liquid biopsies: our experience

Table 3. Concordance of *ALK*, *ROS1*, *RET*, and *MET*Δex14 detection in circulating-free RNA (cfRNA) liquid biopsy vs. tissue by nCounter in absolute number of samples.

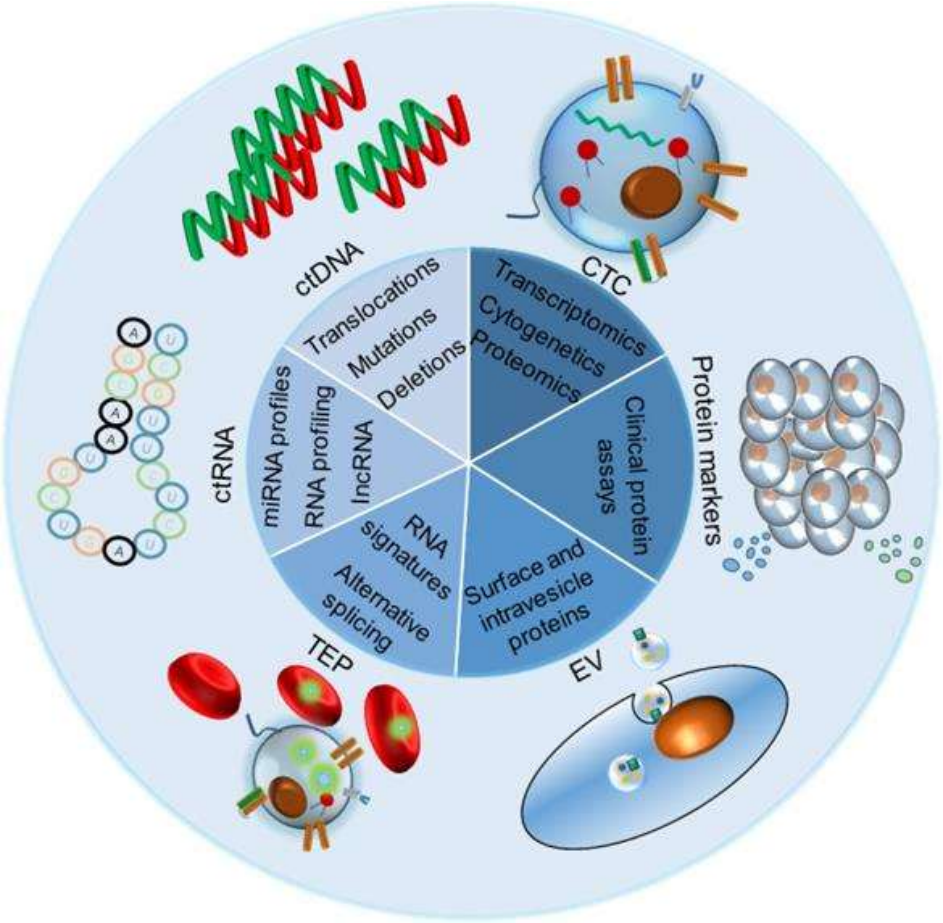
Genes	*ALK*	*ROS1*	*RET*	*MET*Δex14	Overall
No. concordant samples	52	53	51	56	212
No. discordant samples	4	3	5	0	12
Diagnostic sensitivity	71.4% (CI = 45.3–88.3)	67.6% (CI = 35.8–87.9)	58.3% (CI = 30.4–86.2)	100% (CI = 56.6–100)	70% (CI = 54.6–81.9)
Diagnostic specificity	100% (CI = 91.6–100)	100% (CI = 92.4–100)	100% (CI = 91.9–100)	100% (CI = 92.6–100)	100% (CI = 97.8–100)
Concordance	92.85%	94.64%	91.07%	100%	94.41%
Cohen's κ	0.79 (CI = 0.53–1.04)	0.77 (CI = 0.51–1.03)	0.69 (CI = 0.44–0.94)	1 (CI = 0.74–1.27)	0.8 (CI = 0.66–0.92)

Comparison of techniques for fusion detection in liquid biopsy

Characteristics	NanoString Technologies	FoundationOne Medicine	Resolution Bioscience	Guardant Health	ThermoFisher Scientific	ThermoFisher Scientific	NeoGenomics	Roche	RT- PCR
Assay	Custom panel	FoundationOne Liquid CDx	Resolution ctDx Lung	Guardant360 CDx assay	Oncomine Lung cfTNA assay	Ion Ampliseq RNA Fusion Lung Cancer panel	InVisionFirst-Lung	Cobas 4800 system	PCR Taqman assays (Life Technologies)
Input requirements	1 tube of whole blood	two 10-mL tubes of whole blood	two 10-mL tubes of whole blood	two 10-mL tubes of whole blood	1 tube of whole blood	1mL	two 10-mL tubes of whole blood	8 mL of Whole blood	6 mL of whole blood
Type of Sample	cfRNA	cfDNA	cfDNA	cfDNA	cfDNA/cfRNA	cfRNA	cfDNA	cfRNA	cfRNA
Simultaneous detection of SNV/CNV/fusions	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No
Test turnaround time	2 days	15 days	9 days	7 days	4 days	not reported	5 days	1 day	1 day
Reportable range	0.1 - 0.01%	0.125% - 0.5%	1.4%-8%	0.05 - 0.20%	0.02%-2.2% *	not reported	0.06%	not reported	not reported
Sensitivity	70% (95% CI= 55 - 82)	*Datasheet: ALK* 92.9% *Published: 68.4-100%*	81% for fusions	*Datasheet: ALK, RET, ROS1* 83.0%-100% *Published:*18-44%	8% - 100% *	*ALK* (n=9) 64% **	67% for *ALK* and *ROS1*	33.33% (95% CI: 17.3–52.8%)	(n=32) 21.0% in plasma (n= 67) 65.0% in platelets
Specificity	100% (95% CI= 98- 100)	100% for *ALK RET and ROS1 not reported*	Not reported	*Fusions* (n=37) 100% *METΔex14* (n=3) 100%	100% *	100% **	100%	100% (95% CI: 85.8–100%)	100% plasma and platelets
Genes in the panel	*ALK, RET, ROS1* and *METΔex14*	*ALK, RET, ROS1* and *METΔex14*	*ALK, RET, ROS1* and *METΔex14*	*ALK, RET, ROS1* and *METΔex14*	*ALK, RET, ROS1* and *METΔex14*	*ALK*	*ALK, RET, ROS1* and *METΔex14*	*ALK* and *RET*	*ALK*
Study (author, year, reference)	**This manuscript**	(3)	(4)	(5)(6, 7)	(8)	(9)	(10)	(11)	(12)

*No clinical research samples were available to verify the analytical sensitivity.
** Data from extracellular vesicle RNA (EV-RNA).

RNA signatures in liquid biopsies



Wu et al, Theranostics, 2020

-Types of signatures:
--Diagnostic
--Prognostic
--Predictive

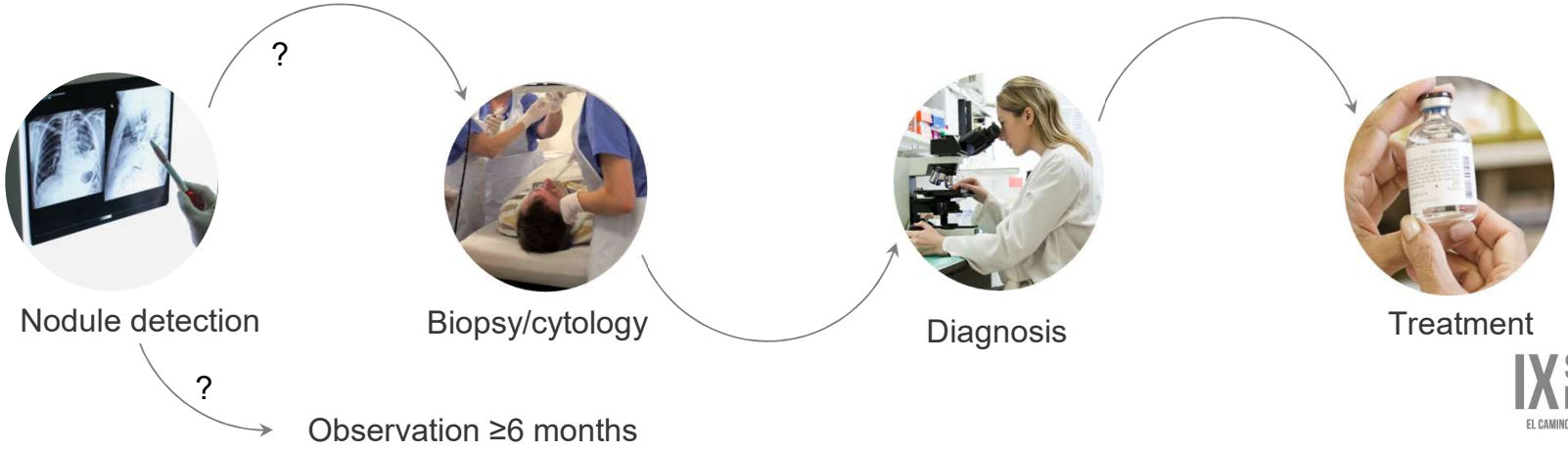
-Types of RNA:
--mRNA
--lncRNA
--miRNA
--circRNA

-Source of RNA
--CTCs
--Circulating RNA (ctRNA)
--Platelet-derived (TEP RNA)
--Exosomes (EV-RNA)

-Several examples in the literature, but not yet in clinical use

Pulmonary nodules

- Last year in Spain 29,638 new cases of the lung cancers diagnosed.
- 80% were diagnosed at advanced stages (IIIB-IV), and have a dismal prognosis, with a median overall survival that does not exceed two years.
- The remaining 20% were diagnosed at early stage (I-IIIA), could undergo surgery and have the potential to be cured.
- Imaging technologies often detect pulmonary nodules of unknown significance.
- In these cases, patients are kept under observation for months (the tumor may grow) or may undergo bronchoscopy (in some cases unnecessary). A guided bronchoscope is capable of sampling 75% of lung nodules larger than two centimeters in size (*SEPAR 2018).*
- A diagnostic test that could help the clinician to differentiate between benign and malignant lesions would be useful in this setting.

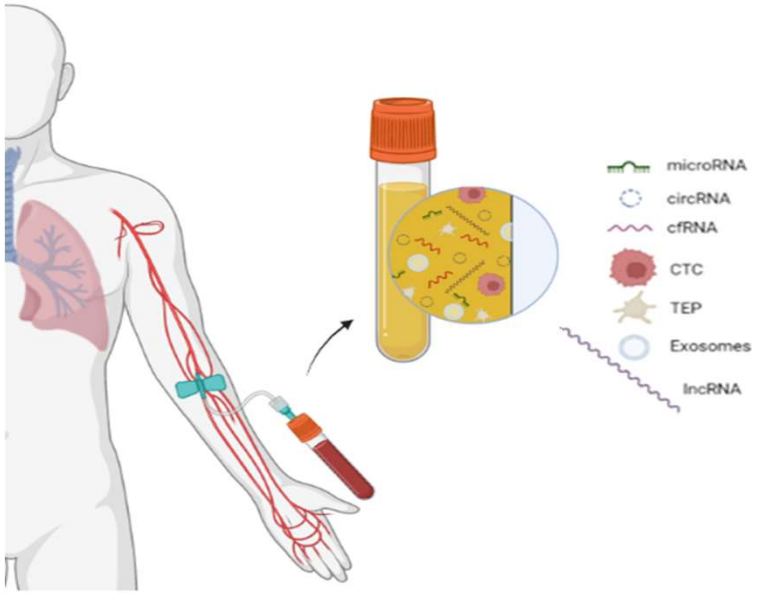


Our experience. Objectives

"The best protection is early detection"

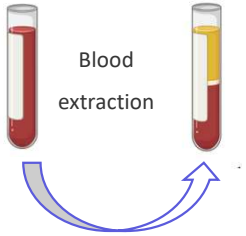
Objective

To develop an **RNA** liquid biopsy-based genetic signature to help the clinician to discriminate benign from malignant nodules in patients with suspicion of lung cancer



Our experience. Methods

SAMPLE PROCESSING <24 HOURS	nCOUNTER FLEX TECHNOLOGY 48-72 HOURS	DATA ANALYSIS



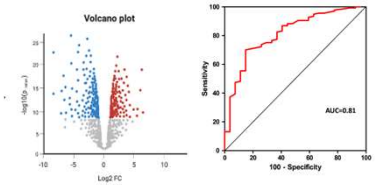
Blood extraction

Plasma processing

RNA purification

Preamplification

- Hybridization
- Purification and immobilization
- Barcode counting



Differential expression and ML analysis

- 1 EDTA tube
- Storage: 2-8ºC for 24 hours

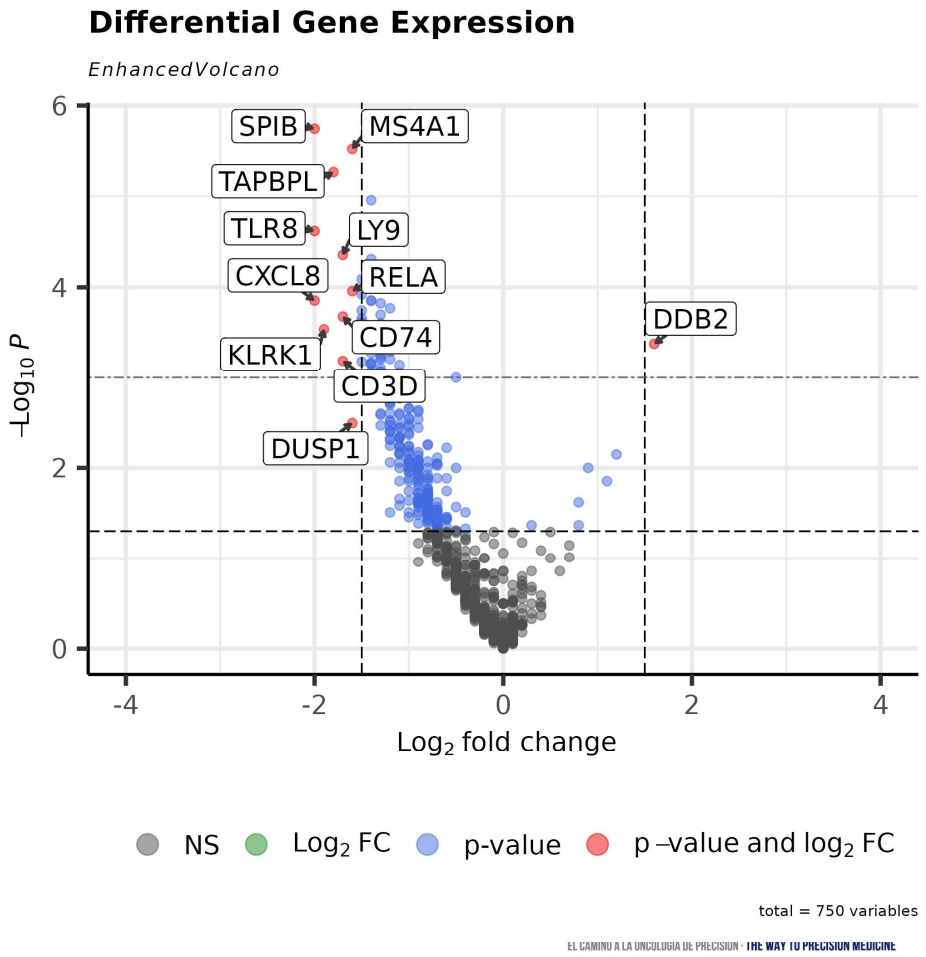
For mRNA signature:

The PanCancer IO 360™ panel is a 770-plex gene expression panel cancer-related inmune responses, micro-enviroment and the tumor.

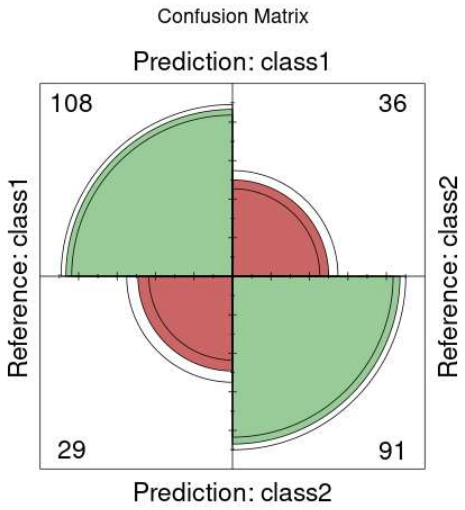
Our experience. Enrolled patients (n=295)

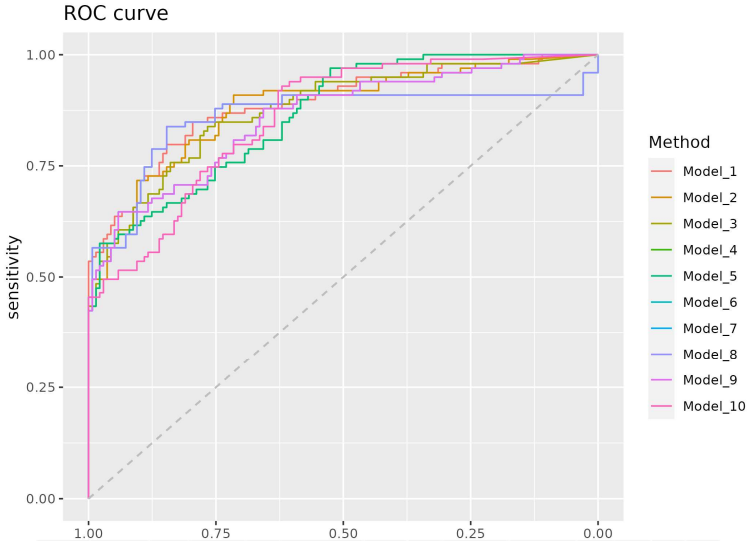
- 149 patients with tumor nodules
- 61 individuals with non tumor lung nodules
- 85 healthy donors

		CANCER (149)	HEALTHY (146)
Gender	Female	70	78
	Male	79	63
	Unknow	0	5
Age	Know	144	139
	Unknow	5	7
	Never	36	66
Smoking status	Current	40	34
	Former	64	31
	Unknow	9	15
Nodules	Yes	149	61
	No	0	85



Our experience. Results



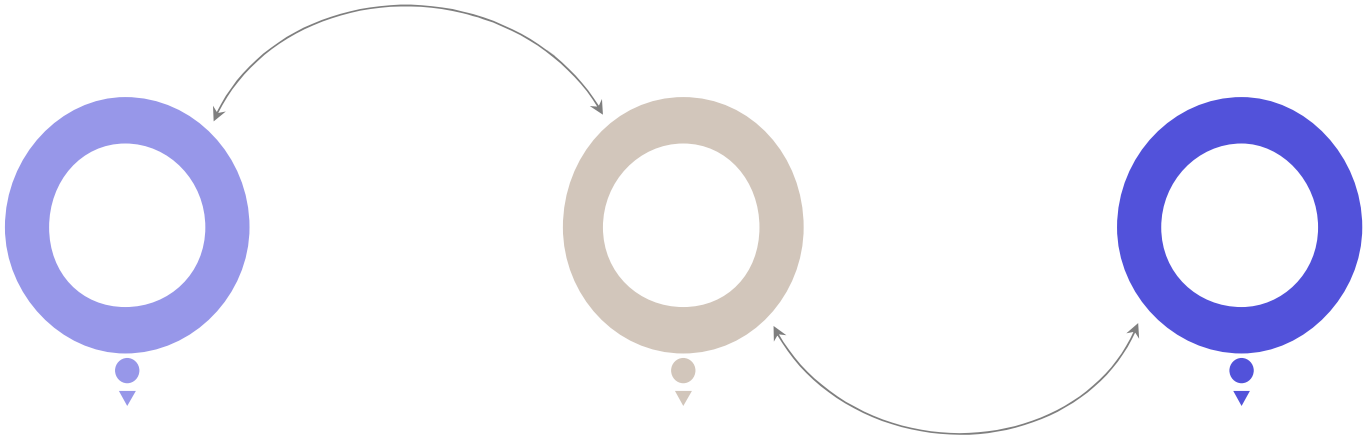


Model	Algorithm	n_var	n_varClin	ROC	Accuracy	Kappa	F1	AccuracyPV	McnemarPV	Sens	Spec	PPV	NPV
Model_1	C50	19	4	0.89	0.81 (0.75 - 0.86)	0.60	0.84	6.80e-14	3.71e-01	0.86	0.74	0.82	0.79
Model_2	C50	20	4	0.88	0.81 (0.75 - 0.86)	0.60	0.84	6.80e-14	2.33e-01	0.87	0.73	0.82	0.80
Model_3	C50	9	4	0.88	0.80 (0.74 - 0.85)	0.59	0.83	6.12e-13	7.70e-01	0.84	0.75	0.82	0.77
Model_4	C50	7	4	0.86	0.76 (0.70 - 0.82)	0.51	0.80	3.47e-09	2.29e-01	0.83	0.67	0.78	0.74
Model_5	C50	7	4	0.86	0.76 (0.70 - 0.82)	0.51	0.80	3.47e-09	2.29e-01	0.83	0.67	0.78	0.74
Model_6	C50	3	4	0.86	0.82 (0.77 - 0.87)	0.63	0.85	2.06e-15	4.49e-02	0.90	0.72	0.81	0.84
Model_7	NN	3	4	0.86	0.79 (0.73 - 0.84)	0.56	0.83	1.36e-11	6.60e-02	0.87	0.68	0.79	0.79
Model_8	C50	3	4	0.86	0.82 (0.77 - 0.87)	0.63	0.85	2.06e-15	4.49e-02	0.90	0.72	0.81	0.84
Model_9	NN	3	4	0.86	0.79 (0.73 - 0.84)	0.56	0.83	1.36e-11	6.60e-02	0.87	0.68	0.79	0.79

11-gene signature (+clinical data)

Area under the ROC curve 0.86-0.89

Our experience. Next steps



Signature Development (n = 300)

Signature training (n = 300) Ongoing

Signature testing / Validation (n = 200)

"Take home" messages

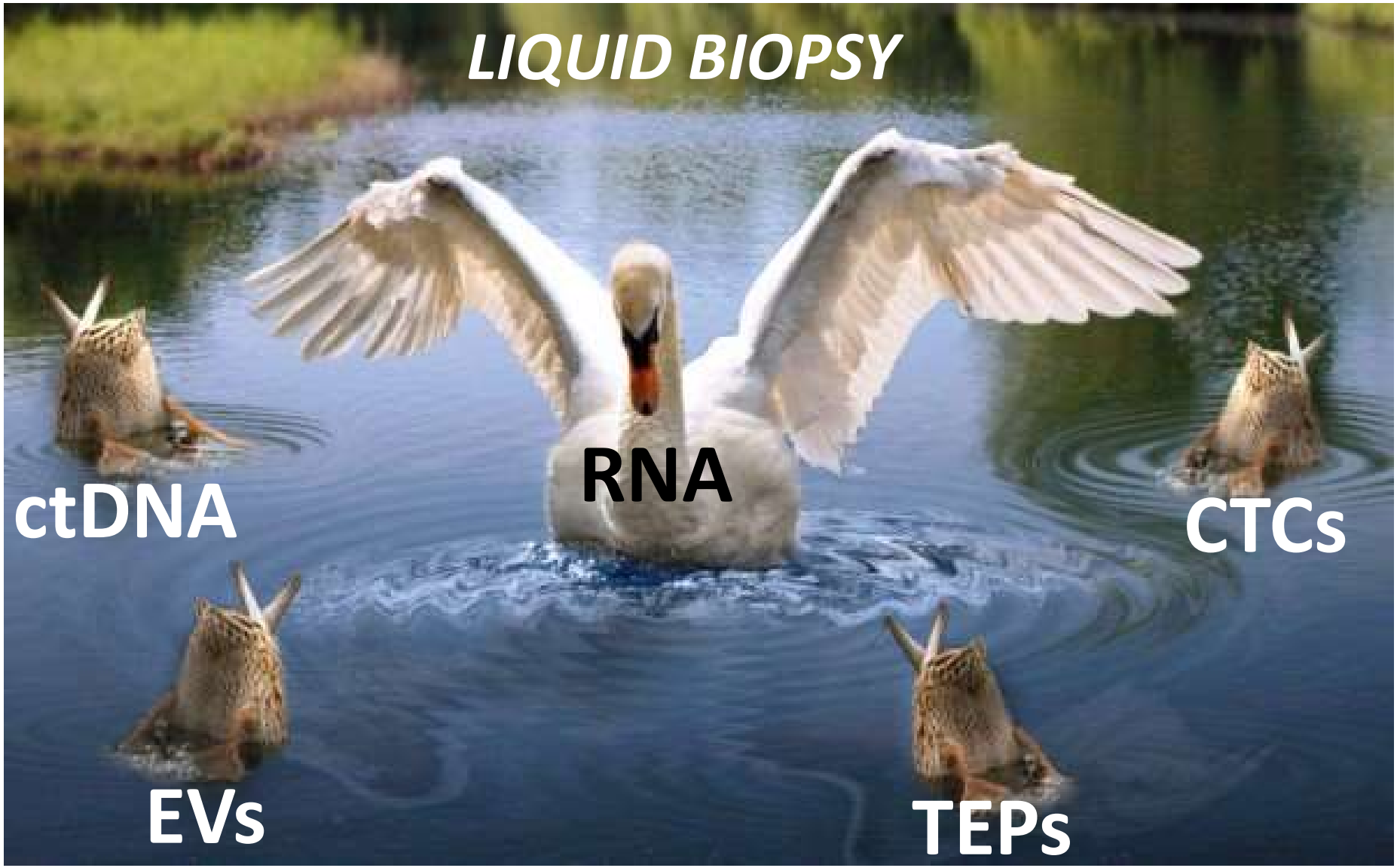
-In cancer patients, tumor RNA (tRNA) can be isolated from CTCs, exosomes, "tumor educated" platelets (TEPs) or plasma (ctRNA)

-RNA isolated from liquid biopsies can be used for (i) detection of clinically relevant fusions and splicing variants, (ii) development of signatures with diagnostic, prognostic or predictive value

-RNA-based techniques should be preferred for the identification of gene fusions and splicing variants. However, they are rarely used in liquid biopsies. Development and validation are currently under way.

-Several signatures based on RNA isolated from liquid biopsy sources have been published, particularly for cancer detection, and more are in development.

-However, such signatures are not (yet) in clinical use


LIQUID BIOPSY
ctDNA
RNA
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¡GRACIAS!

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