I.O.V.
ISTITUTO ONCOLOGICO VENETO

Monitoraggio dinamico delle mutazioni di KRAS nel cfDNA e risposta alla terapia sistemica in pazienti con neoplasie polmonari

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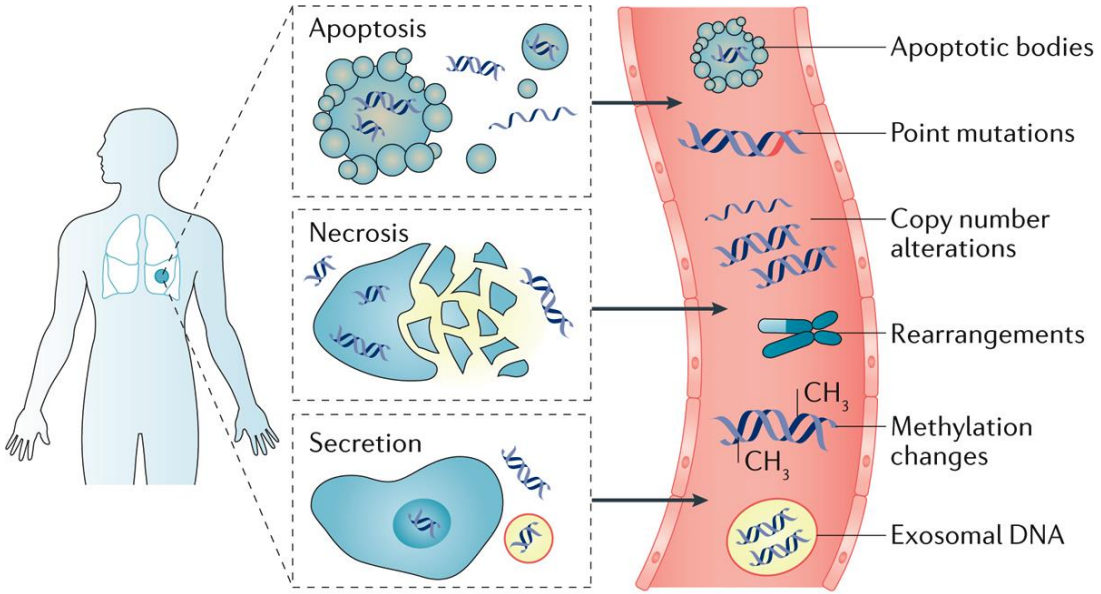
MEDICAL NEEDS & PRIORITIES

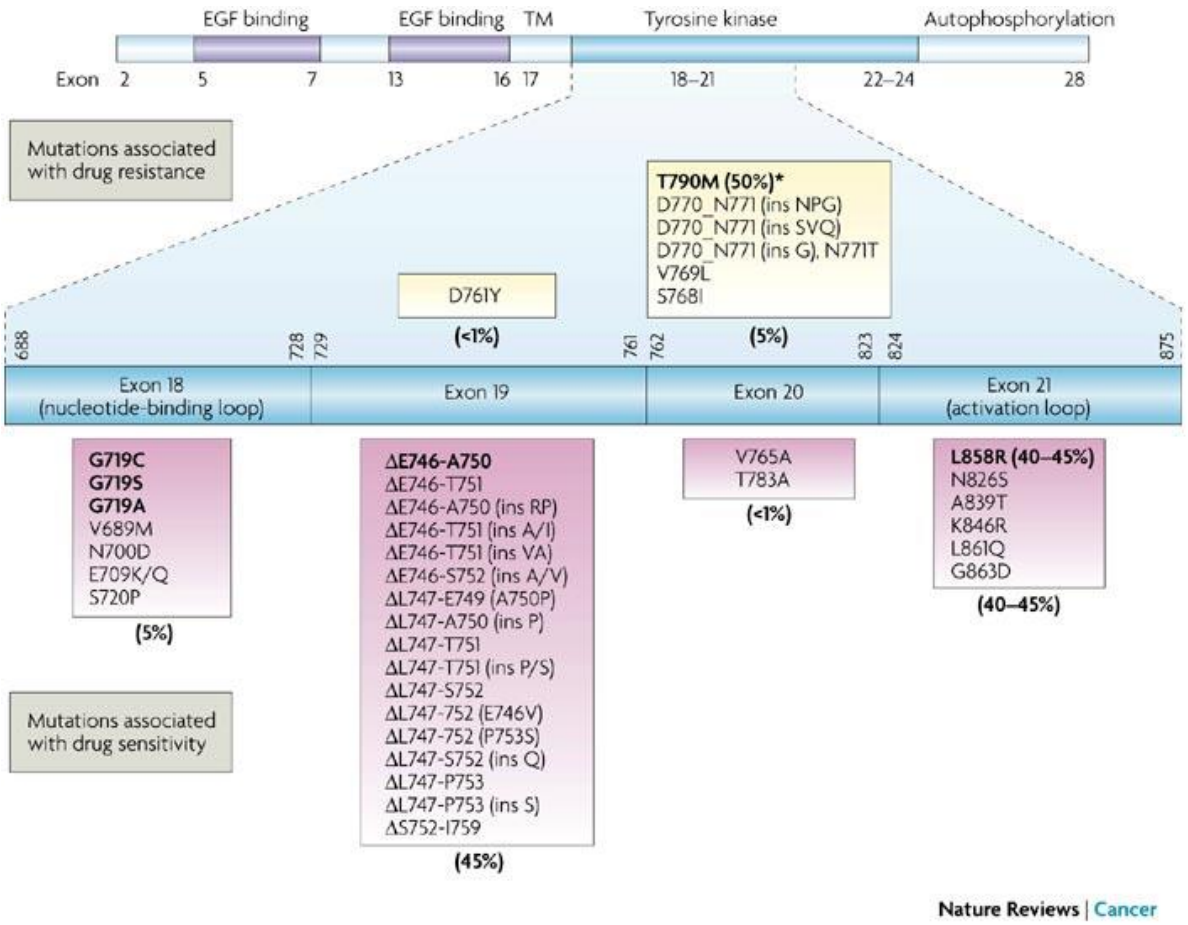
- **In most lung cancer patients, limited information about genetic alterations in tumor cells at relapse is available**
- **Genetic characterization of tumor DNA can disclose important therapeutic clues**

Circulating tumor DNA (ctDNA) is a fraction of cell free DNA (cfDNA)



Nature Reviews | Cancer

EGFR mutations and cfDNA: a success story



Tissue biopsy

PROS

- ✓ The source of DNA is well-defined
- ✓ Easy to keep at RT

CONS

- ✓ Not always feasible
- ✓ Samples can be inadequate
- ✓ Expensive
- ✓ Risky

It is often not possible to perform multiple longitudinal biopsies from the same patient or parallel sampling from primary tumor and metastasis.

Liquid biopsy

PROS

- ✓ It can be repeated
- ✓ Minimally invasive, high compliance
- ✓ Comparatively cheap

CONS

- ✓ cfDNA has short half-life
- ✓ Tumor DNA cannot be directly measured
- ✓ Concentration of cfDNA typically very low

Diaz LA Jr and Bardelli A, J Clin Oncol 2014

www.impactjournals.com/oncotarget/ Oncotarget, Advance Publications 2016

Guide to detecting epidermal growth factor receptor (*EGFR*) mutations in ctDNA of patients with advanced non-small-cell lung cancer

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JOURNAL OF CLINICAL ONCOLOGY ASCO SPECIAL ARTICLE

Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review

Jason D. Merker, Geoffrey R. Oxnard, Carolyn Compton, Maximilian Diehn, Patricia Hurley, Alexander J. Lazar, Neal Lindeman, Christina M. Lockwood, Alex J. Rai, Richard L. Schilsky, Apostolia M. Tsimberidou, Patricia Vasalos, Brooke L. Billman, Thomas K. Oliver, Suanna S. Bruinooge, Daniel F. Hayes, and Nicholas C. Turner

Blood drawing: EDTA versus Streck tubes

(pre-analytical issues)

EDTA K2

1. Plasma should be obtained within 2 h
2. @ 1600-2000g 10 min +4°C
3. @ 20000g 10 min +4°C
4. Make plasma aliquots and store at -80°C

STRECK

1. Plasma can be obtained within 7 d
2. Tube inversion at time of blood drawing very important
3. Same protocol as for EDTA tubes

N=6								
		EDTA K2 tubes			Streck tubes			
	Mutation found:	Ct Control mix	Ct mut	Delta Ct	Time from drawing	Ct Control mix	Ct mut	Delta Ct
E104	non mut	28,65	/	/	6 d	27,44	/	/
E106	ex 19 del	27,61	30,59	2,98	6 d	24,91	29,81	4,9
E110	L858R	27,26	27,51	0,25	2 d	27,7	27,66	-0,04
	S768I		26,61	-0,65			26,7	-1
P12	ex 19 del	25,75	27,67	1,91	0 d	28,58	29,54	1,96
P16	ex 19 del	27,64	26,03	1,61	6 d	27,28	28,05	0,77
	T790		31,13	3,49			32,69	5,41
E109	non mut	27,61	/	/	2 d	28,08	/	/

TECHNICAL PERFORMANCE OF THE ASSAYS USED TO DETECT EGFR MUTATIONS IN cfDNA (analytical validity)

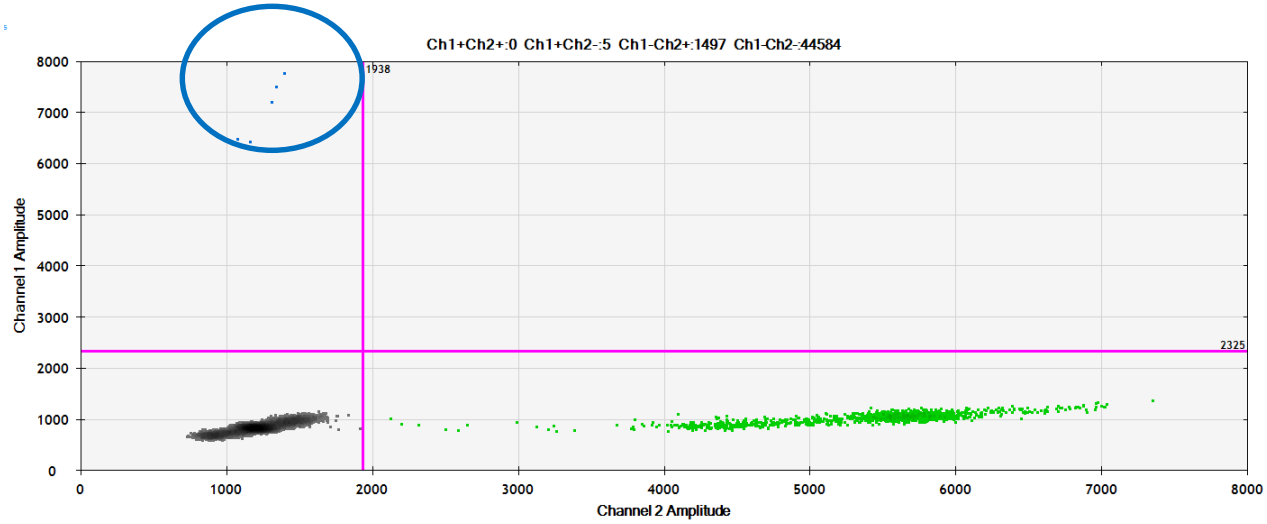
cfDNA analysis of patients recruited within Emulating trial (IOV)

	Real-time PCR (DIATECH)	ddPCR** (Biorad)
Identification of EGFR exon 19 del / L858R mutations	44.4% (44/99)	55.5% (55/99)
Identification of T790M mutation in PD patients	42% (13/31)	56% (17/31)

** cut-off recommended: 3 single positive droplets

EMULATING TRIAL: example of ddPCR output

Patient #54: EGFR exon 21 (L858R) mutation detected in tissue at diagnosis
Mutation NOT detected in cfDNA by real-time PCR (Diatech easy EGFR kit)
Mutation detected in cfDNA by ddPCR (variant allele fraction VAF= 0.33%)



Should liquid biopsy for EGFR be repeated?

Patients negative for EGFR testing on plasma, undergoing clinical progression

	No mutation	Sens +/ T790 -	Sens +/ T790+
Repeated samples (n=35)	60%(21/35)	20% (7/35)	20% (7/35)

Clinical case (female, 72 Y/O, never smoker, diagnosis 03/2014, surgery, pT2N2, relapse 03/2015)

Courtesy of Alessandro Dal Maso & c.

Disease burden	LFN Bone	**Pleural effusion** LFN Bone	Pleural effusion LFN Bone	**Liver** Pleural effusion LFN Bone	Liver Pleural effusion LFN Bone	**Liver (new lesions)** Pleural effusion **LFN** Bone	Liver Pleural effusion LFN Bone
Liquid biopsy		Negative		**L858R**		**T790M** L858R	
Tissue biopsy		No biopsable lesions		Not done (patient's choice)		Non-diagnostic	
Time	03/2015	10/2016	01/2017	09/2017	10/2017	06/2018	08/2018
Treatment	Gefitinib		Carboplatin-pemetrexed		Docetaxel		Osimertinib
Response	PR	PD	SD	PD	SD	PD	Ongoing

CONCLUSIONS

- EGFR cfDNA testing is a routine assay in molecular diagnostics
- Sensitivity of available CE IVD assays is about 0.5% mutated allele in cfDNA
- Analytical validity is ~55% and needs to be improved
- Repeated cfDNA testing can lead to detection of occult mutations
- Clinical utility of EGFR mutation detection in cfDNA demonstrated (Osimertinib)

BACKGROUND

Non-oncogene addicted NSCLC

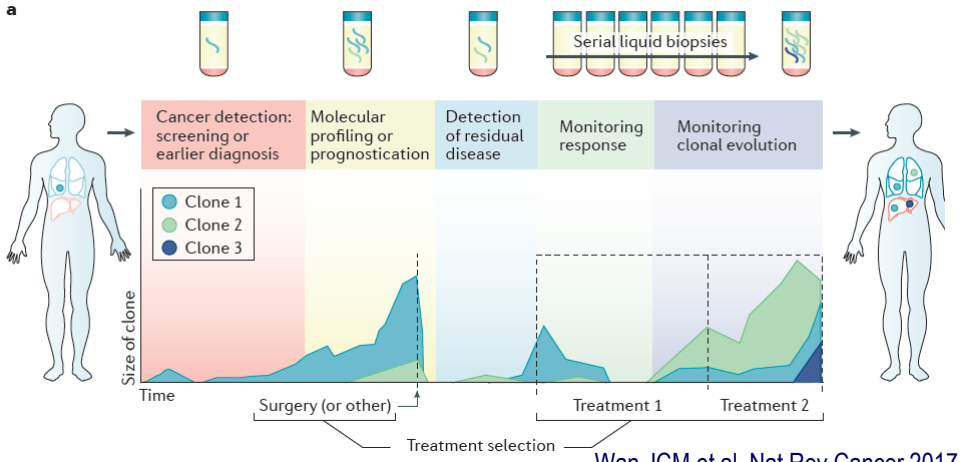
Immune Checkpoint Inhibitors (ICIs) have changed clinical perspective

- PD-L1 is a marker for selecting patients for first-line ICI monotherapy (pembrolizumab, ≥ 50%)
- Combination treatment (chemotherapy plus ICIs) demonstrated to be superior to chemotherapy in first-line setting (independently of PD-L1)
- Duration of clinical benefit from ICIs is highly heterogeneous and detrimental effects have been described

Reck M et al, NEJM 2016; Gandhi L et al, NEJM 2018; Socinski SA et al, NEJM 2018; Paz-Ares L et al, ASCO 2018

Liquid biopsy: potentialities

- Prompt detection of tumor genetic alterations
- Dynamic monitoring of tumor burden
- Insights on tumor heterogeneity



Wan JCM et al, Nat Rev Cancer 2017

AIM of the MAGIC-1 study

- **To assess the validity of liquid biopsy in clinical practice for non oncogene addicted NSCLC**
  primary end-point: sensitivity in plasma at baseline for **KRAS mutations**

- **To investigate the predictive role of longitudinal analysis of sentinel mutations in plasma**

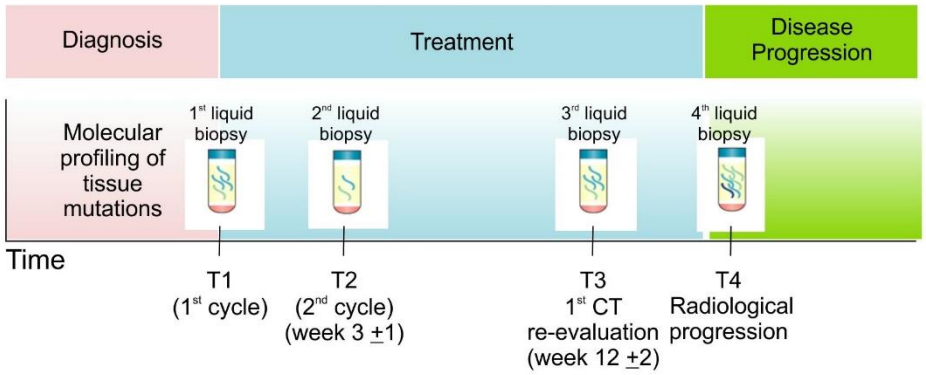
MAGIC study: Monitoring Advanced NSCLC through plasma Genotyping: Clinical feasibility and application- Validation 1- Methods

Genetic screening-
(tissue-Sequenom MassARRAY®/NGS)

↓

Detection and quantification
of specific genetic alterations
(plasma-ddPCR/NGS)

→



- Prospective enrolment of advanced NSCLC patients consecutively treated with chemotherapy or ICIs (January 2017-April 2018) and tissue genotyping
- *KRAS*-mutated patients (in tissue): relative quantification of *KRAS* mutation in plasma by using ddPCR and evaluation of fractional abundance of mutated allele (MAFA)
- Assessment of sensitivity of *KRAS* mutation in plasma at baseline
- Association of *KRAS* mutation in plasma at baseline and during treatment with outcome: radiological response (RECIST v1.1), progression-free survival (PFS), overall survival (OS)

BASELINE LIQUID BIOPSY: RESULTS

162 patients enrolled
(*EGFR-ALK-ROS1* wt)

↓

87 tested in tissue

↓

44 positive in tissue

↓

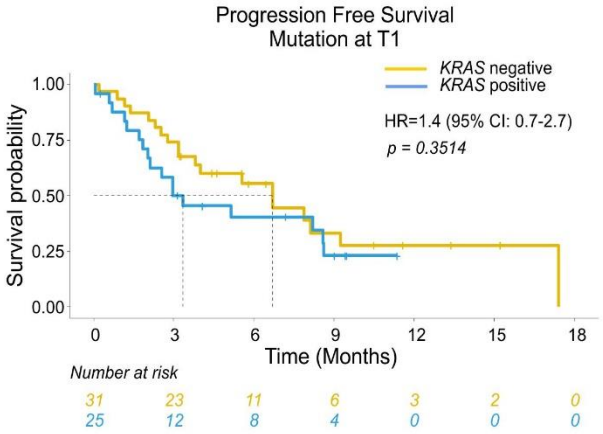
42 tested in plasma

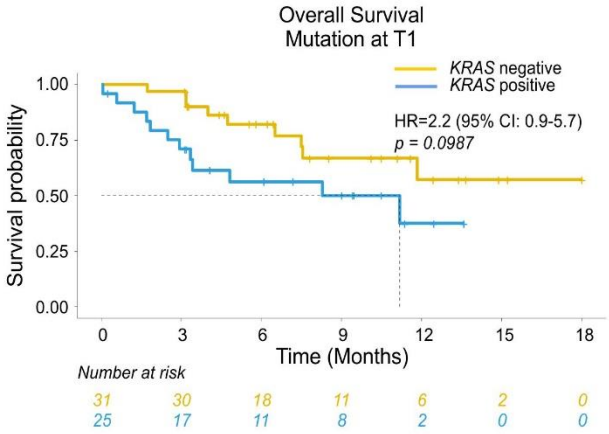
↓

19/42 positive in plasma
(45%; 95%CI: 29.8-61.3)

The presence of *KRAS* mutation in plasma at baseline:

- Was not associated with tumor burden
- Was not statistically associated with outcome (trend for worse outcome)





LONGITUDINAL EVALUATION OF *KRAS* MUTATION IN PLASMA

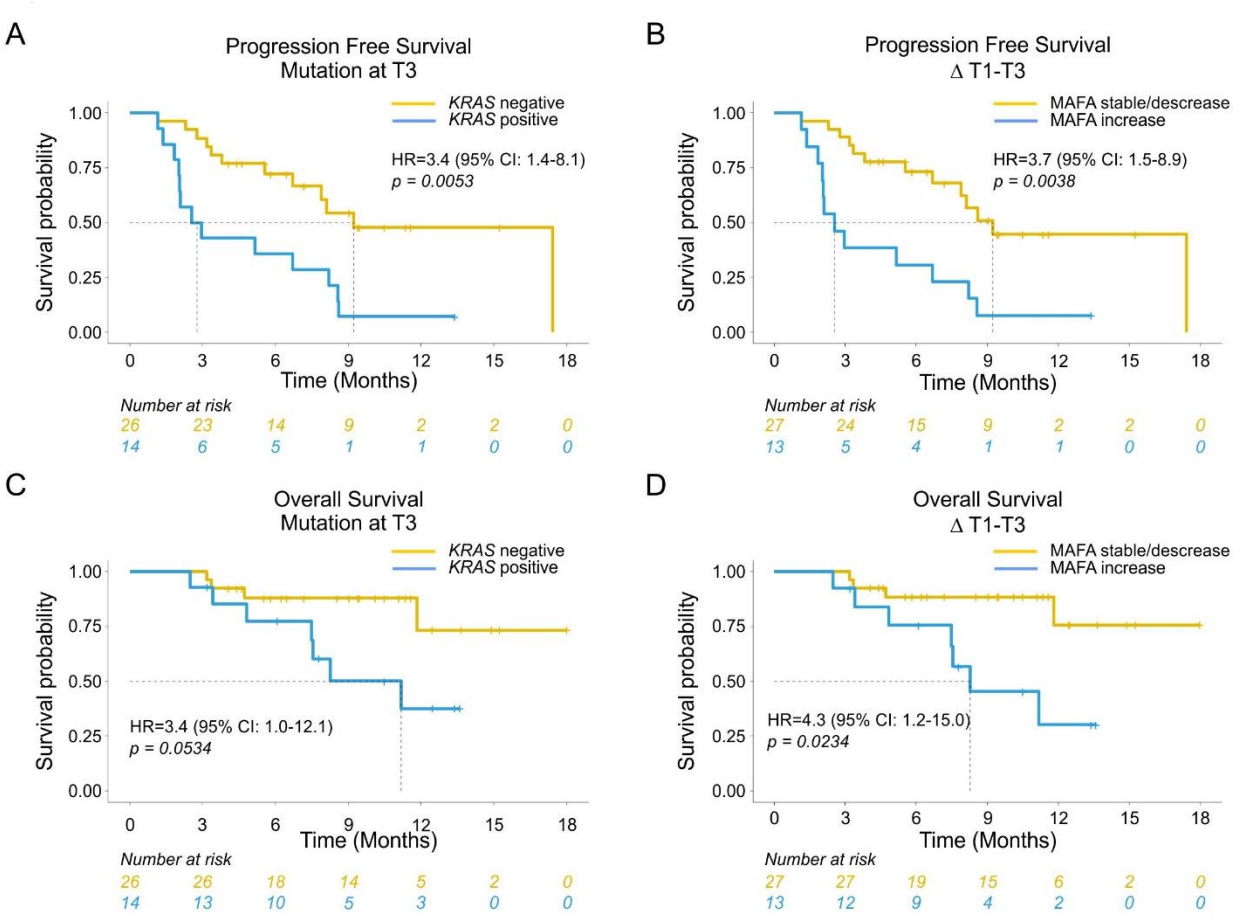
The presence of *KRAS* mutation at T2 predicts the risk for PD as best radiological response

		PROGRESSIVE DISEASE	ODDS RATIO (OR)	95%CI	p-value	AUC	Adjusted* OR (PD)	95%CI	p-value
Mutation T1 (baseline)	No	9/26	1				1		
	Yes	11/21	2.1	0.7-6.2	0.1602	0.59 (0.4-0.7)	1.9	0.6-5.9	0.2919
Mutation T2 (3±1 weeks)	No	6/23	1				1		
	Yes	11/20	3.5	1.2-10.0	0.0189	0.65 (0.5-0.8)	**4.1**	1.3-13.1	**0.0179**
Mutation T3 (12±2 weeks)	No	4/26	1				1		
	Yes	9/14	10.4	2.2-45.0	0.0033	0.75 (0.6-0.9)	**10.9**	2.0-60.6	**0.0064**

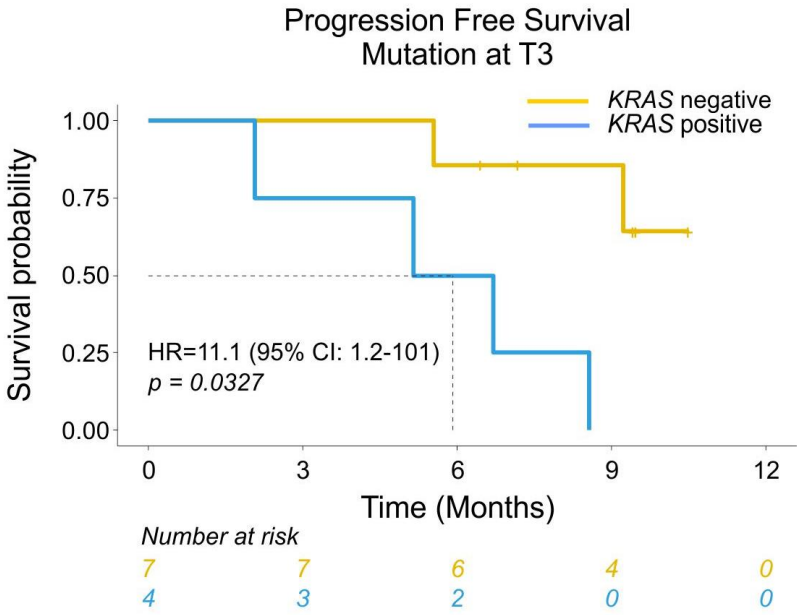
*smoking and number of metastatic sites

LONGITUDINAL EVALUATION OF *KRAS* MUTATION

The presence of *KRAS* mutation at T3 (w12±2) and its increase T1-T3 (Δ) predict worse outcome

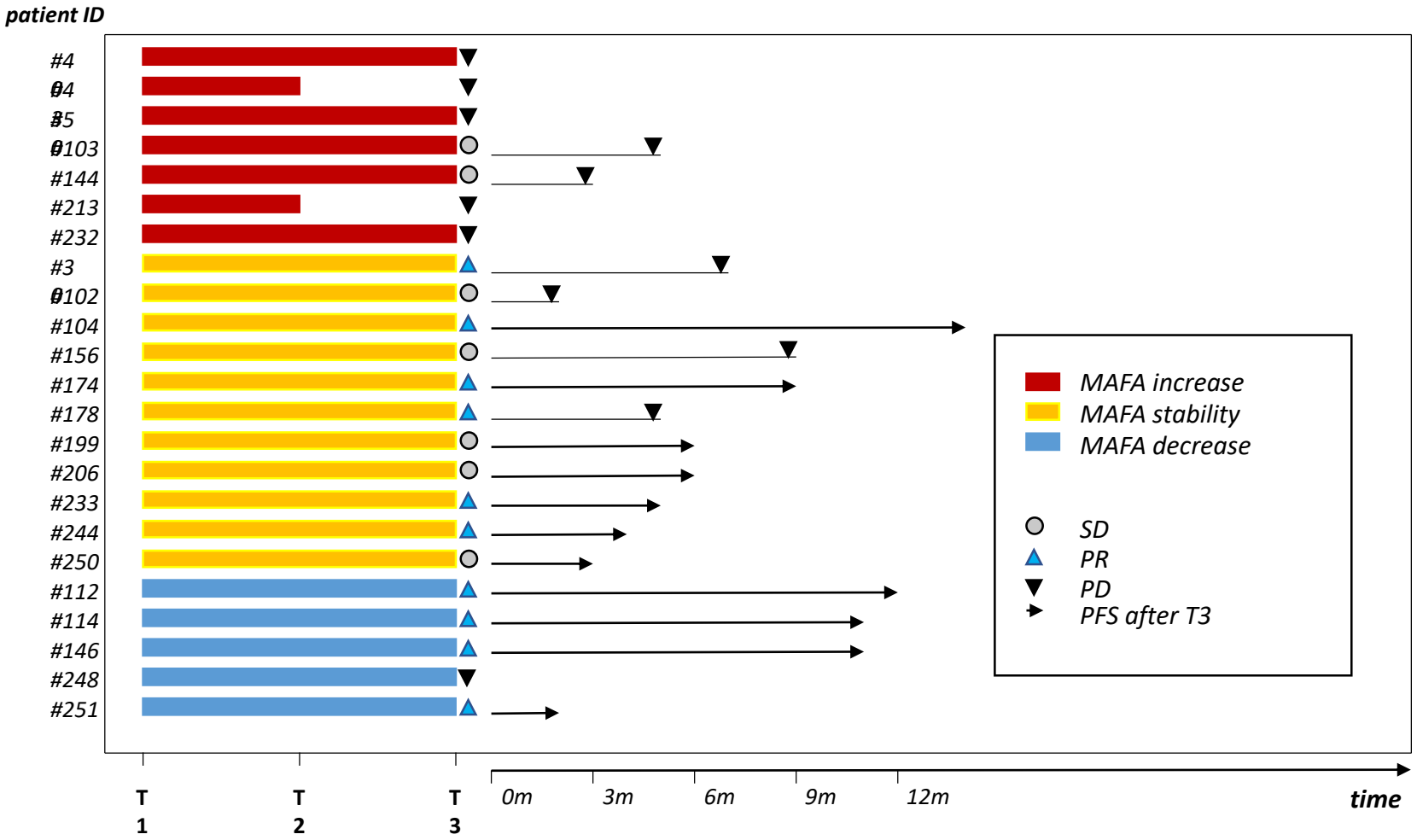


LONGITUDINAL EVALUATION OF *KRAS* MUTATION AND IMMUNOTHERAPY



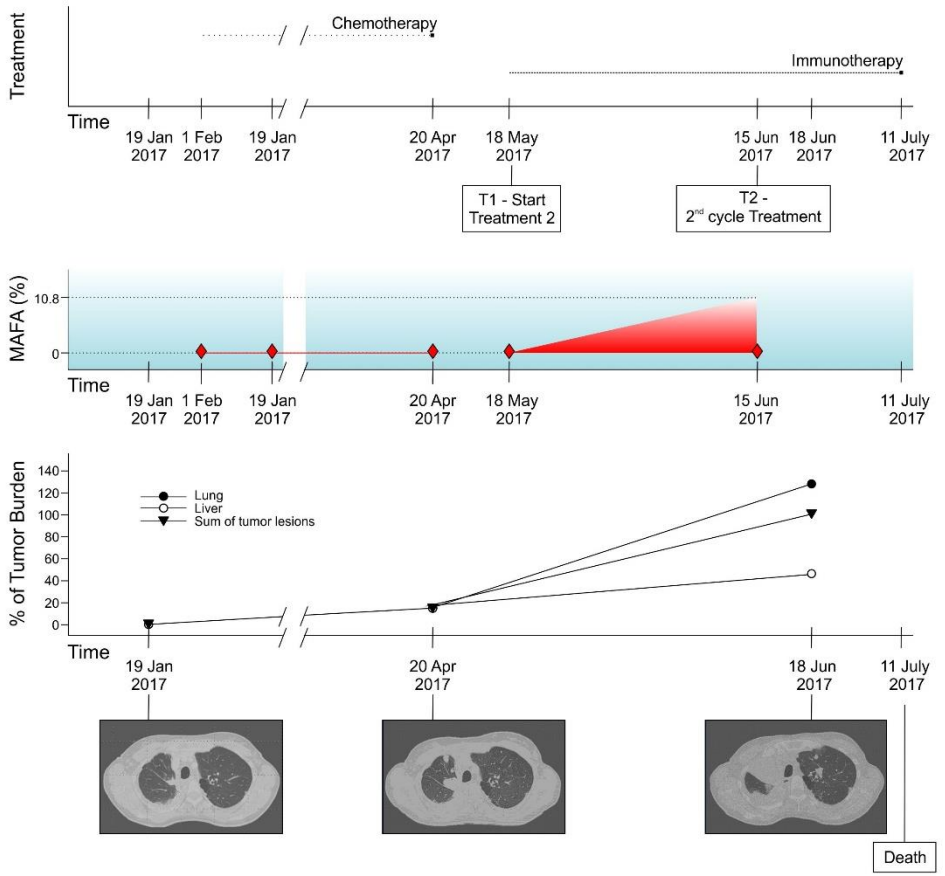
The presence of *KRAS* mutation at T3 (w12±2)
and its increase T1-T3 (Δ)
predict worse PFS

LONGITUDINAL EVALUATION OF *KRAS* MUTATION AND IMMUNOTHERAPY



LONGITUDINAL EVALUATION OF *KRAS* MUTATION AND IMMUNOTHERAPY: HYPER-PROGRESSION

One case of hyper-progression*:
Increase 0-10% in MAFA T1-T2 (4 weeks)



*Hyperprogression is observed in 10-15% of NSCLC patients treated with ICIs
Champiat S et al, CCR 2017
Ferrara R et al, Jama Onc 2018

CONCLUSIONS

- Baseline *KRAS* mutation in plasma was not statistically associated with outcome
- *KRAS* mutation detected after 3±1 weeks from the start of treatment predicts radiological response
- *KRAS* mutation detected at T3 (12±2 weeks) predicts shorter PFS
- Increase in fractional abundance of *KRAS* mutation from baseline to T3 (12±2 weeks) predicts shorter PFS and OS

PERSPECTIVES

- More frequent monitoring in plasma to assess the best timing to predict long-term clinical benefit

- Ongoing ddPCR and NGS analysis to confirm the results by analysing other “sentinel mutations” in plasma

- Ongoing expansion cohort in ICIs treated patients to confirm the predictive value of dynamic analysis of sentinel mutations in plasma and potential application in predicting hyper-progression

- Clinical utility: exploitation of dynamic KRAS mutation levels in cfDNA for patient stratification purposes (ICIs versus ICIs/CT)

Acknowledgments

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