Monitoring circulating tumor DNA during immunotherapy in NSCLC patients by targeted NGS: preliminary data on the CORELAB project.

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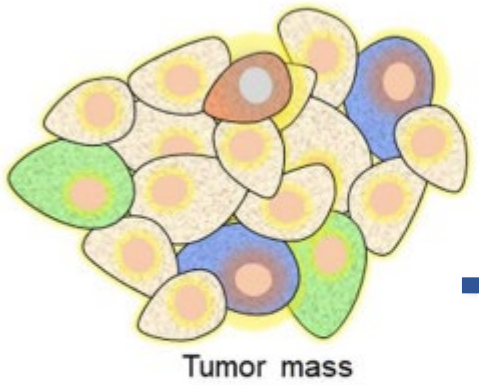
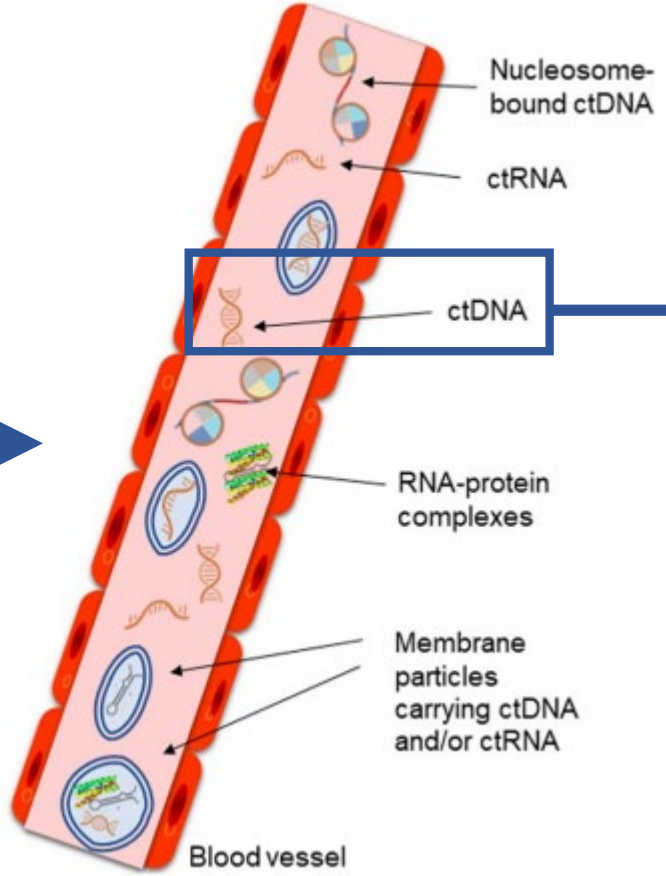
Liquid biopsy and circulating tumor DNA (ctDNA)

Liquid biopsy: A laboratory test done on a sample of blood, or other body fluids to look for cancer cells, DNA, RNA, or other molecules released by tumor cells into a person's body fluids *(NCI)*.





ctDNA is the part of Circulating Cell free DNA (cfDNA) originating from tumor cells:

- carries tumor-specific genetic features

Stejskal et al. Molecular Cancer (2023) 22:15

ESMO reccommendation:

- ctDNA testing if tissue not available or when fast turnaround time is needed for urgent therapeutic decision making
- Lung cancer: ctDNA genotyping recommended in treatment-naïve cancer patients and resistance upon prior TKIs.

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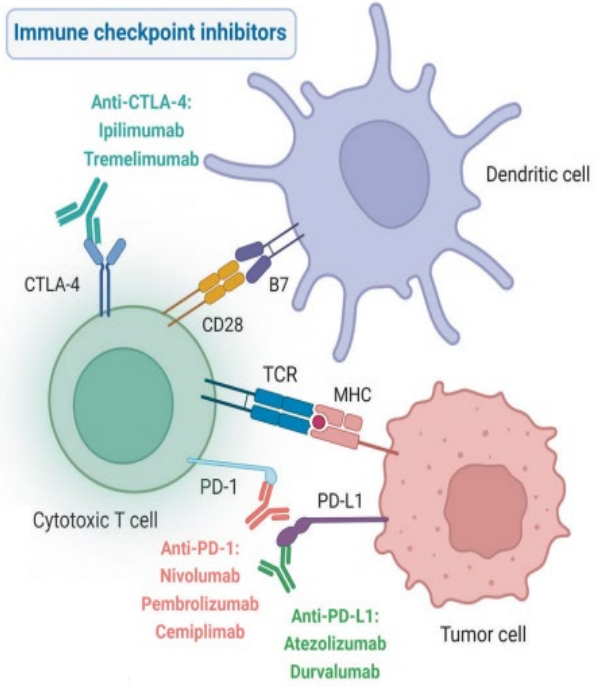
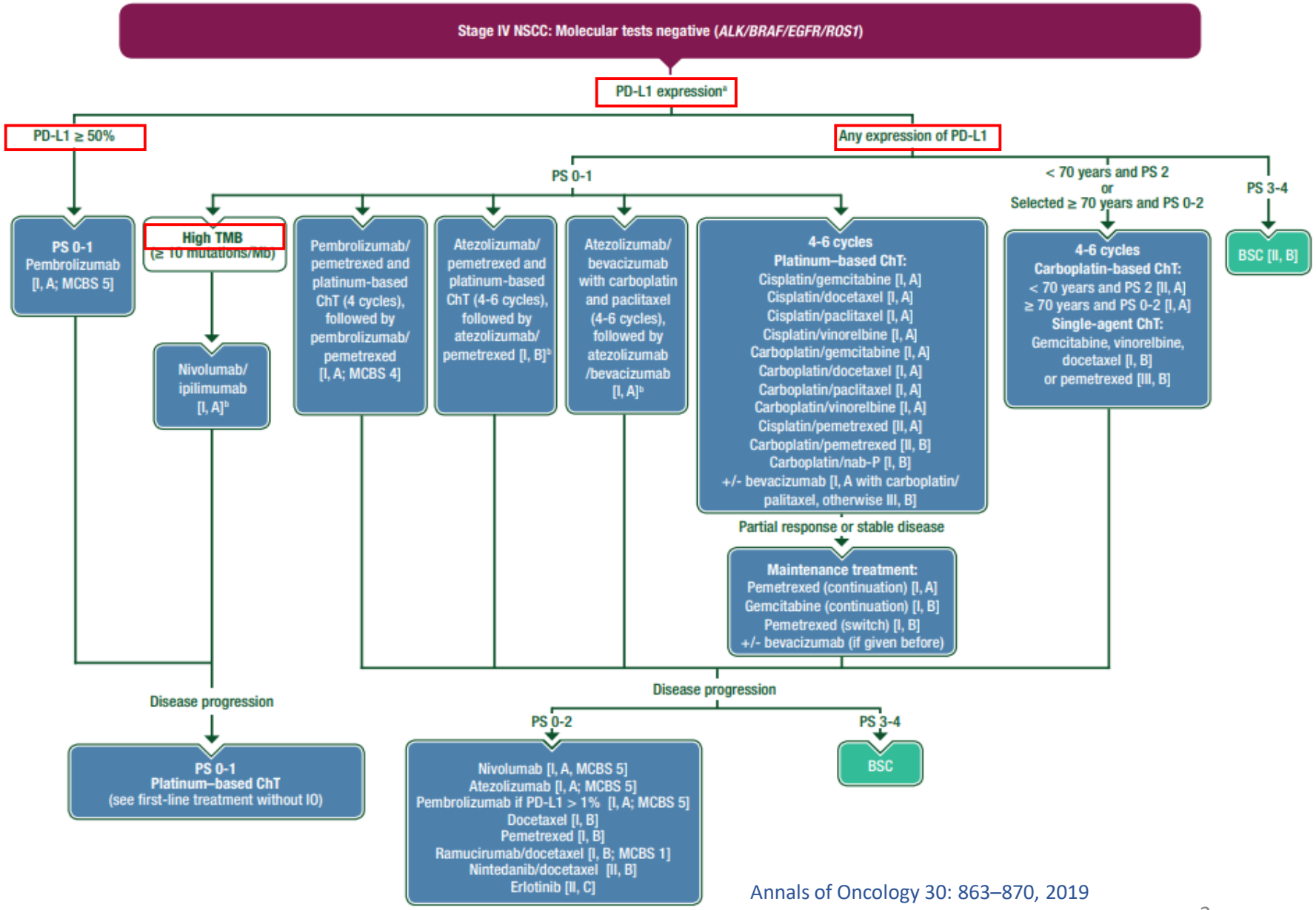
Immunotherapy in Non-Small Cell Lung Cancer (NSCLC)



Cell Death and Disease (2023) 14:230

- PD-L1
- TMB

Novel predictive immunotherapy biomarkers are still required



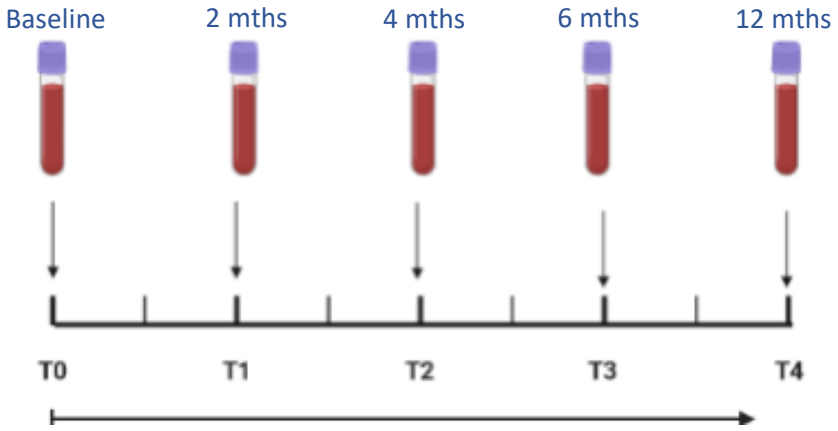
Annals of Oncology 30: 863–870, 2019

The CORELAB project

The project includes patients with diagnosis of advanced stage (III-IV) Non-Small Cell Lung Cancer, candidate to therapy with Immune Check point Inhibitors (ICI) as I line or II line treatment. Patients are monitored from the start of ICI up to 12 months or until disease progression. Standard and novel targets (both from tissue and blood) are analyzed during these follow-up to identify efficient predictive biomarkers of immune check point inhibitors in advanced NSCLC.

Experimental design and aim

Blood based liquid biopsy at multiple time points, plasma cfDNA isolation and cfDNA targeted NGS analysis



Assay	Genes	Selected SNV hotspots
Oncomine™ Lung cfDNA Assay	*ALK, BRAF, EGFR, ERBB2, KRAS, MAP2K1, MET, NRAS, PIK3CA, ROS1,* and *TP53*	>150 hotspots including: *EGFR*: T790M, C797S, L858R, Exon 19 del *KRAS*: G12X, G13X, Q61X *BRAF*: V600E *ALK*: Exon 21-25 *PIK3CA*: E545K, H1047R, E542K

Results

Cases and samples:

50 NSCLC patients

↓

- 40 T0-T1 paired samples
- 10 T0 sample only

Mutational status:

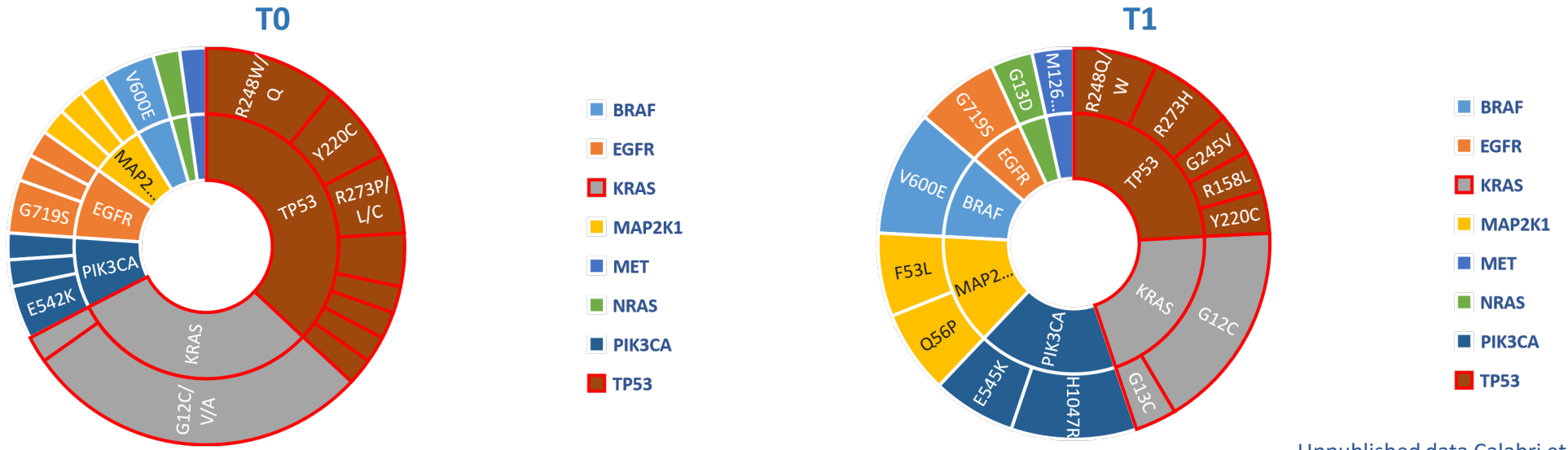
Pre-treatment (T0)

- 19/50 (38%) no mutation detected in cfDNA
- 31/50 (62%) mutation detected in ctDNA: 22/50 one variant, 9/50 more than one variant

On-treatment (T1-after 2 months):

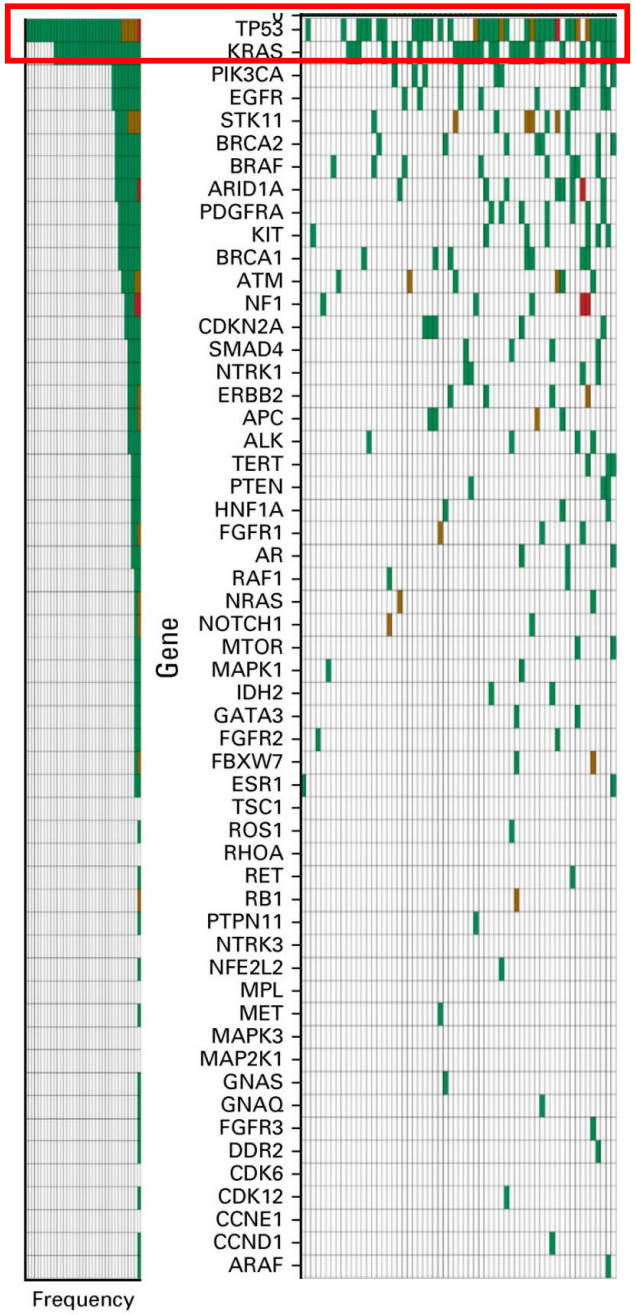
- 26/40 (65%) no mutation detected in cfDNA
- 14/40 (35%) mutation detected in ctDNA: 6/40 one variant, 8/40 more than one variant

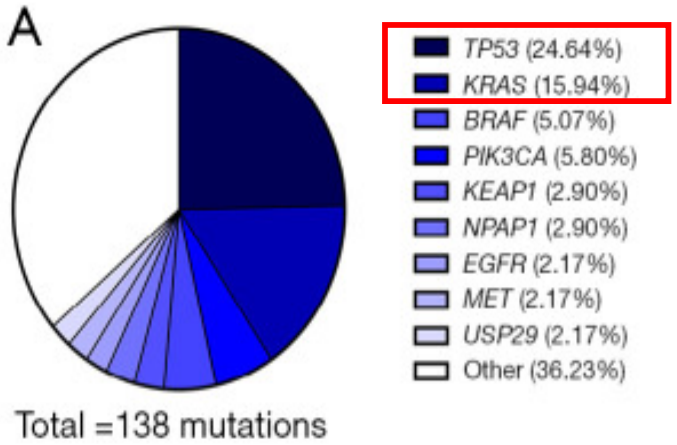
ctDNA – altered genes and variants pre-treatment and on-treatment



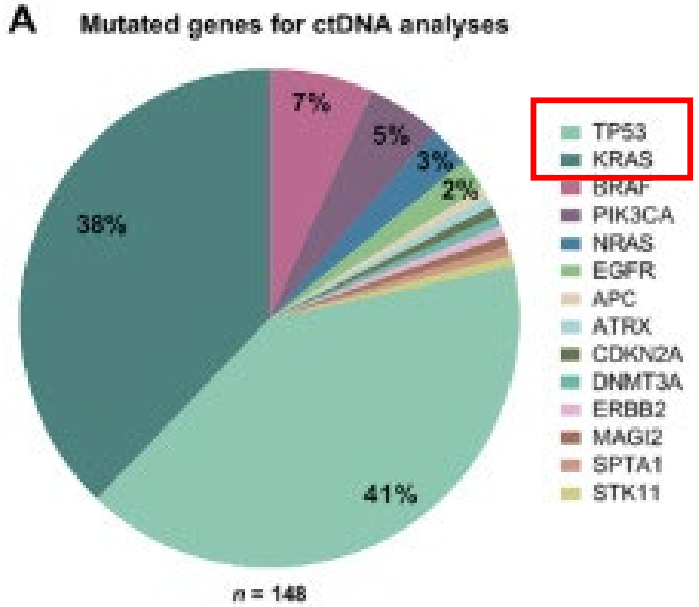
Unpublished data Calabri et al.

Results





Transl Lung Cancer Res. 2023 May 31; 12(5): 971–984



Cancer Res Commun. 2022 Oct; 2(10): 1174–1187

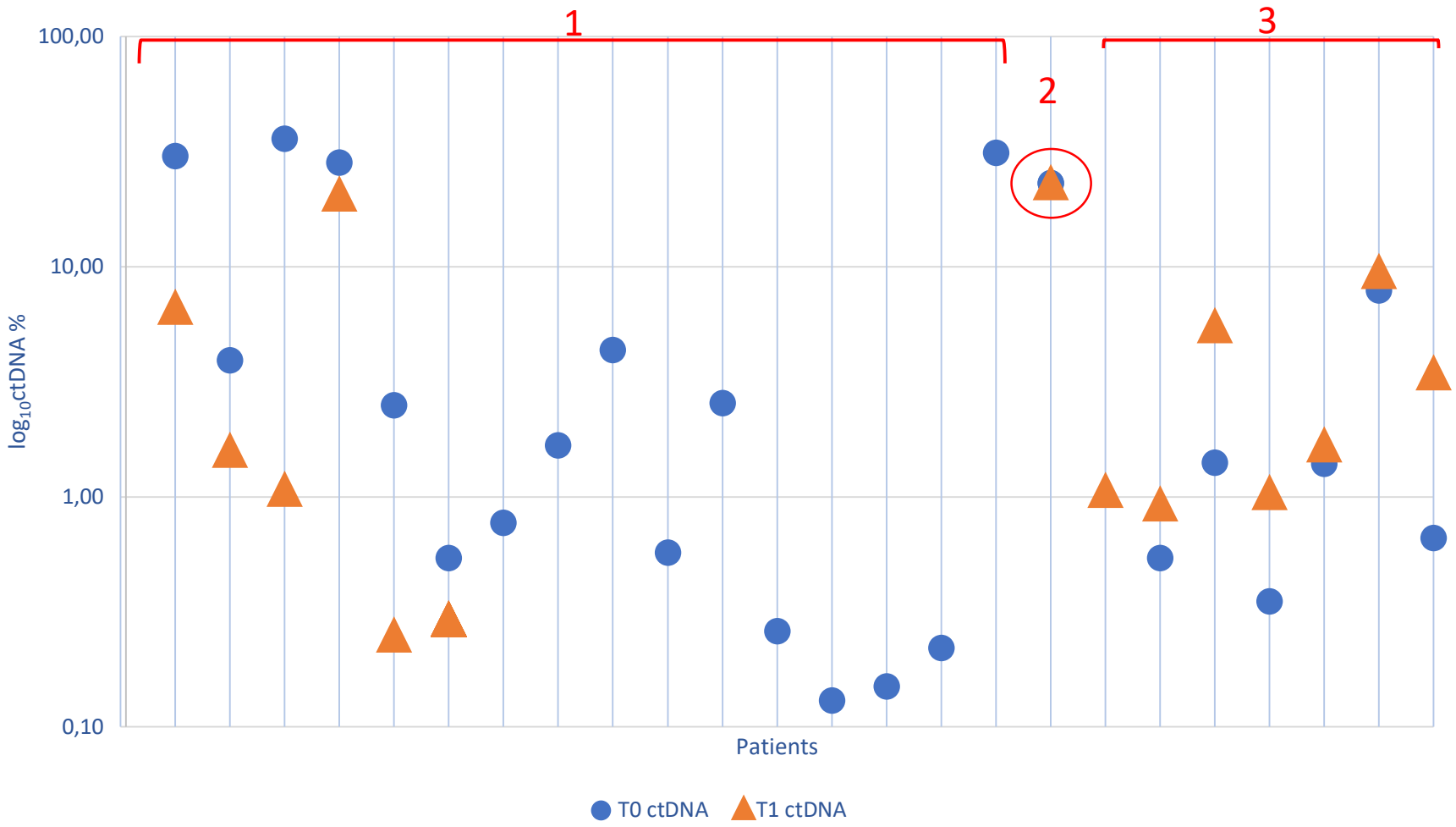
JCO Precision Oncology no. 5 (2021) 510-524

Results

ctDNA – dynamics between T0 and T1

- 16/40 (40%) patients no detected mutation both at T0 and at T1 on cfDNA

1. 16/40 (40%) patients reduction in ctDNA% (10 complete disappearance)
2. 1/40 (2,5%) patient stable ctDNA% between the two checkpoints
3. 7/40 (17,5%) patients rise in ctDNA % (1 with no detected mutation at baseline)



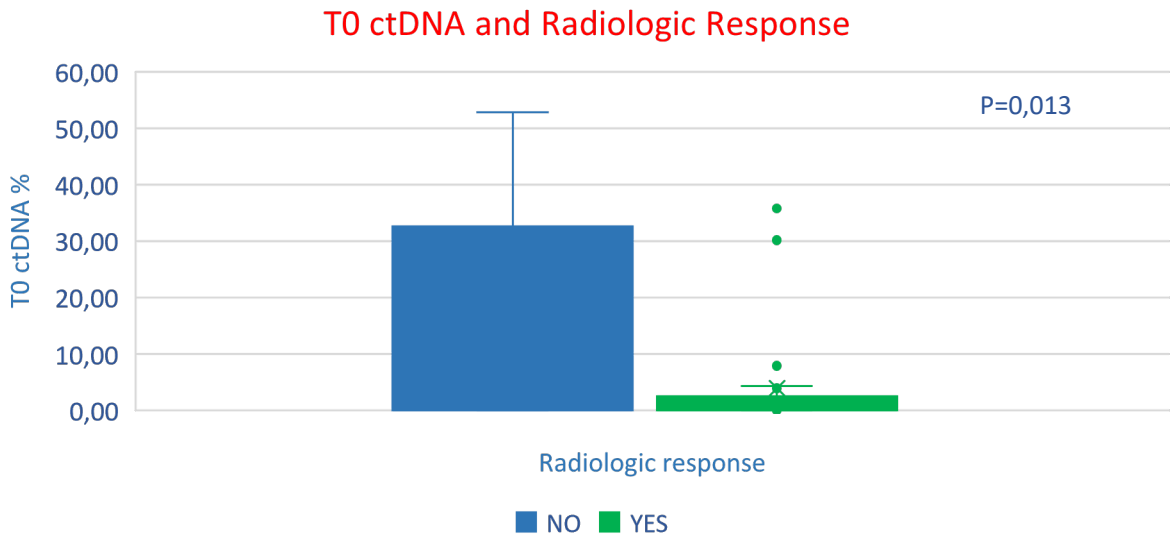
* ctDNA quantitative evaluation (ctDNA%) was estimated making the VAFs' sum of different mutations observed in the same sample

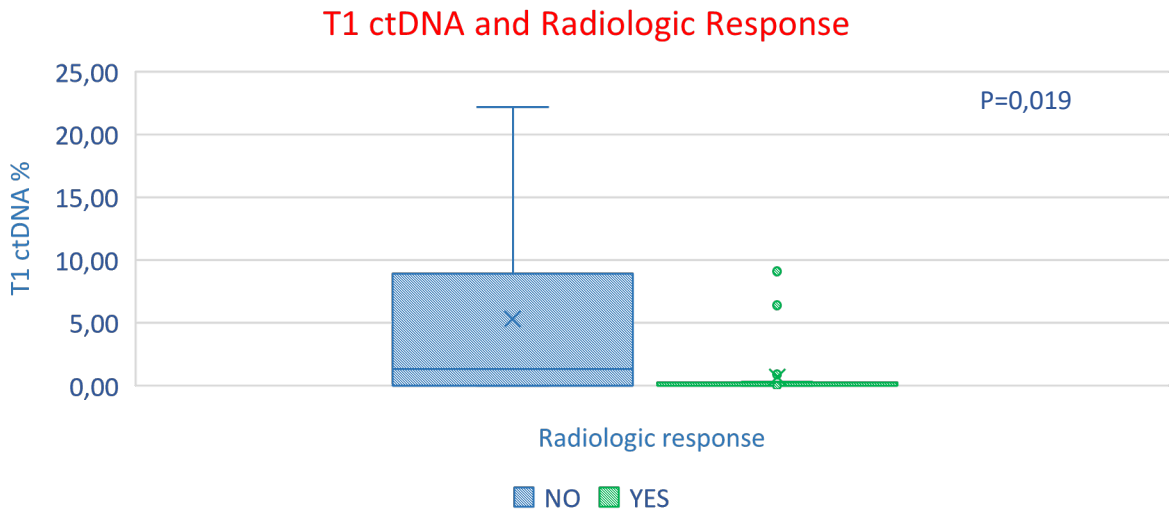
Unpublished data Calabri et al.

Results

ctDNA – T0 and T1 correlation with clinical outcome: Radiologic Response

	No radiologic response	Radiologic response
N° of patients	19	31





Higher ctDNA% is associated with worst radiologic outcome

* ctDNA quantitative evaluation (ctDNA%) was estimated making the VAFs' sum of different mutations observed in the same sample

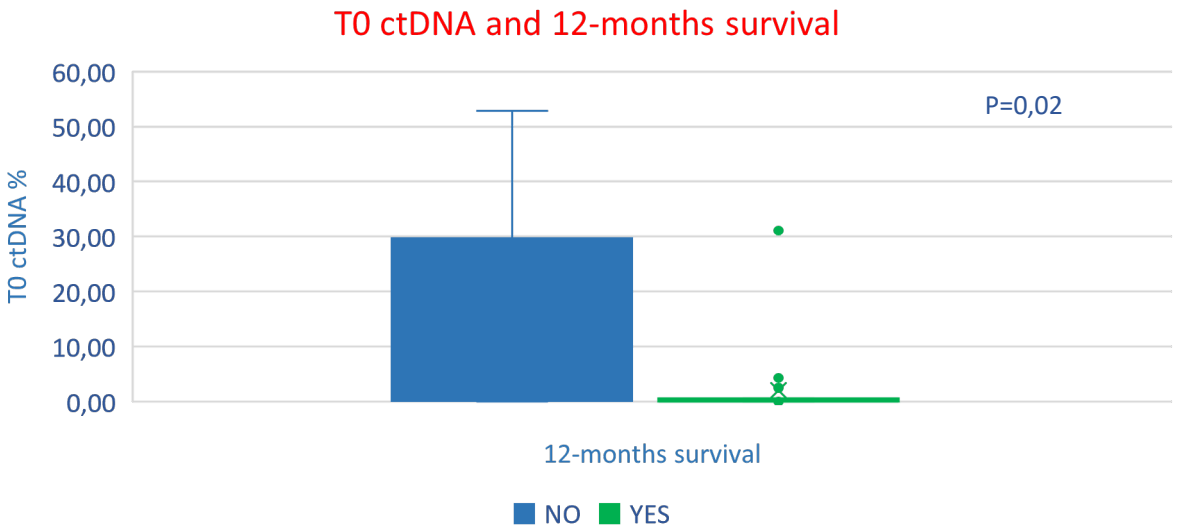
Unpublished data Calabri et al.

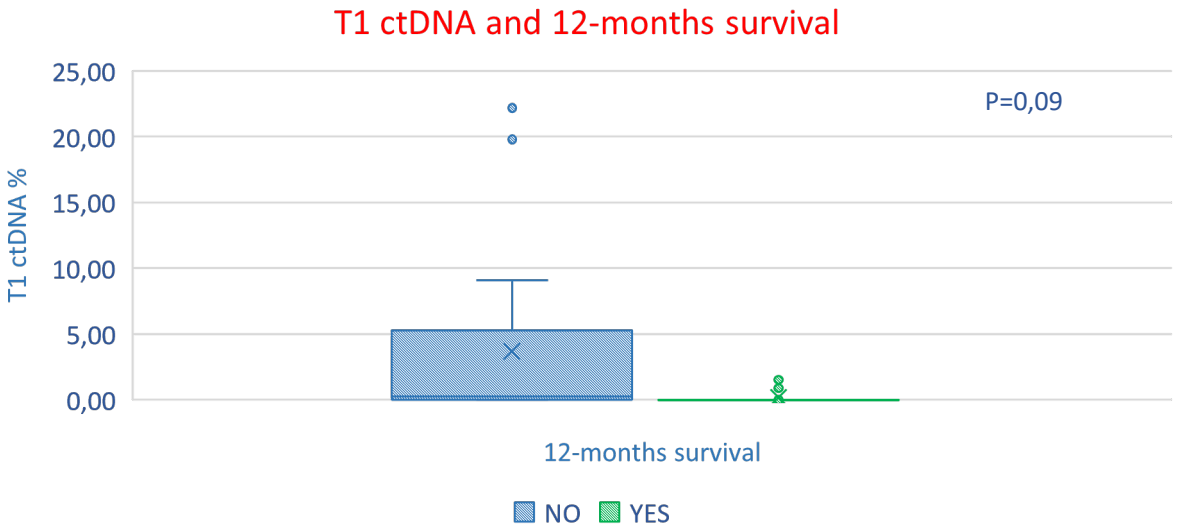
Results

ctDNA – T0 and T1 correlation with clinical outcome: 12-months survival

	No 12-months survival	12 months survival
N° of patients	28	22

12-months survival was evaluated from T0 (immunotherapy start)





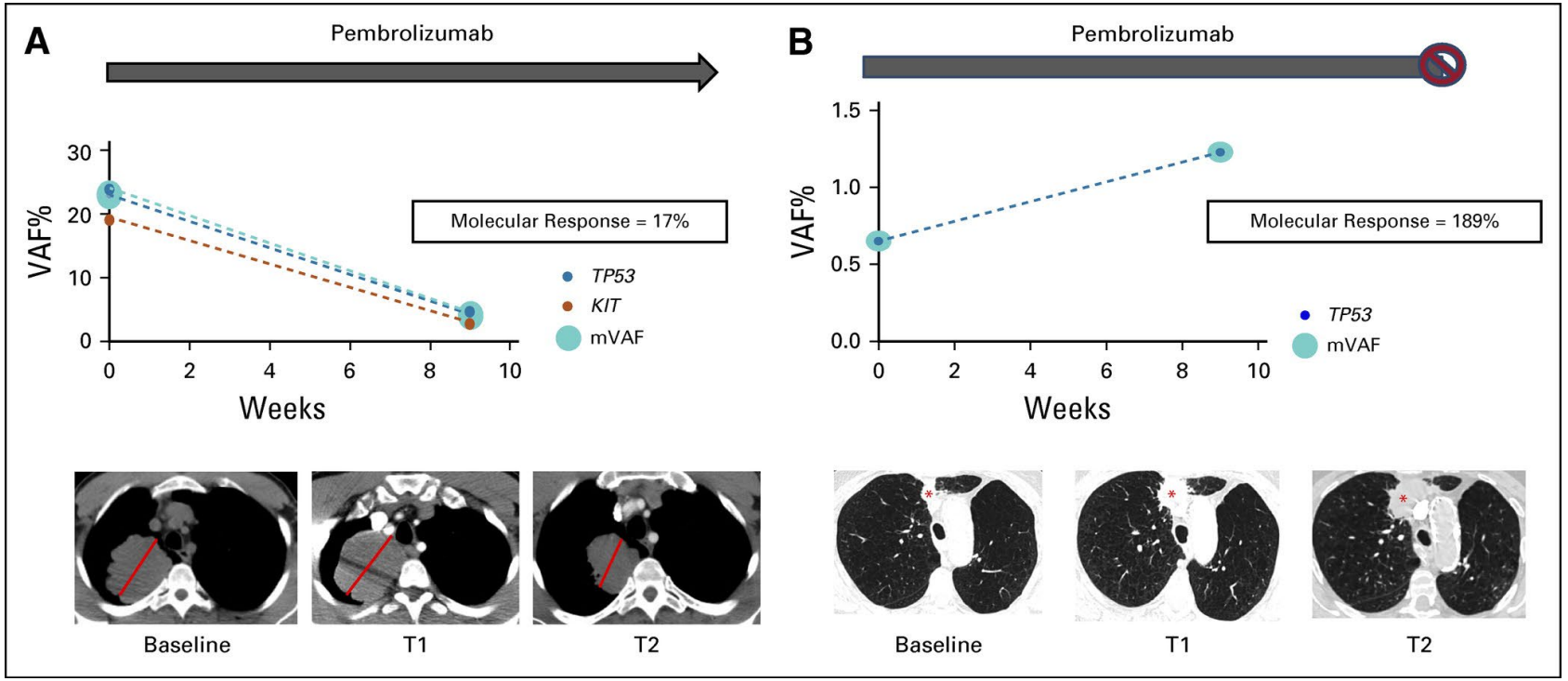
Higher ctDNA% is associated with worst survival outcome

* ctDNA quantitative evaluation (ctDNA%) was estimated making the VAFs' sum of different mutations observed in the same sample

Unpublished data Calabri et al.

Results

ctDNA – longitudinal monitoring



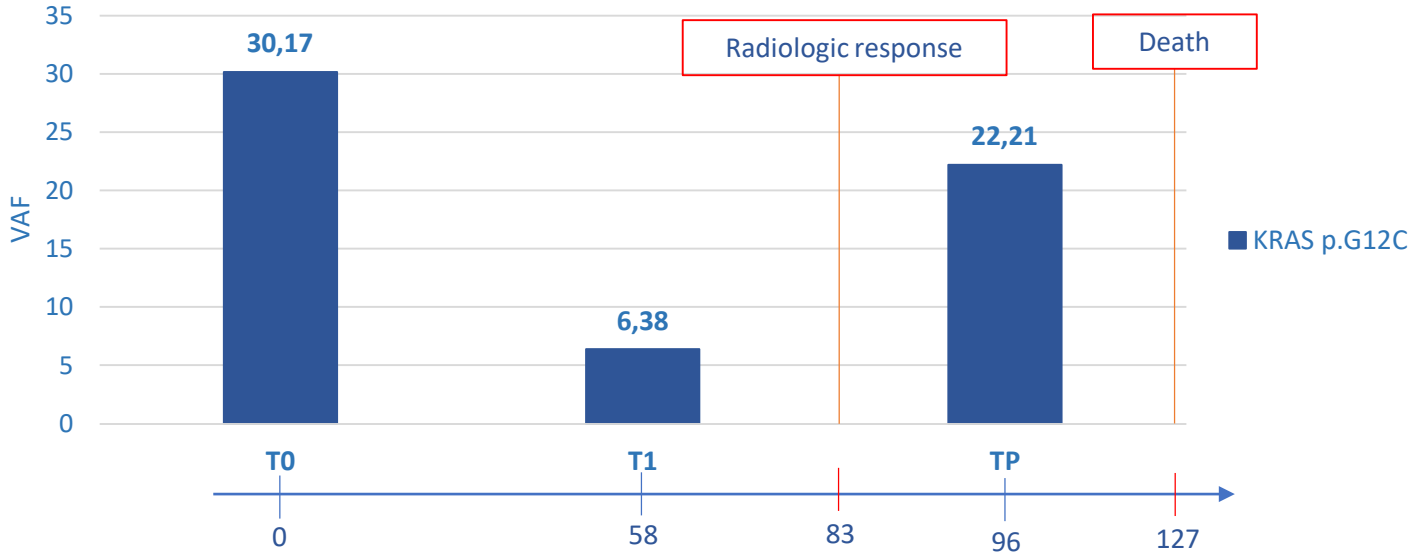
JCO Precision Oncology no. 5 (2021) 510-524.

Results

ctDNA – longitudinal monitoring

* Variant Allele Frequency (VAF) was used to quantify each single mutation found in a sample

(1)



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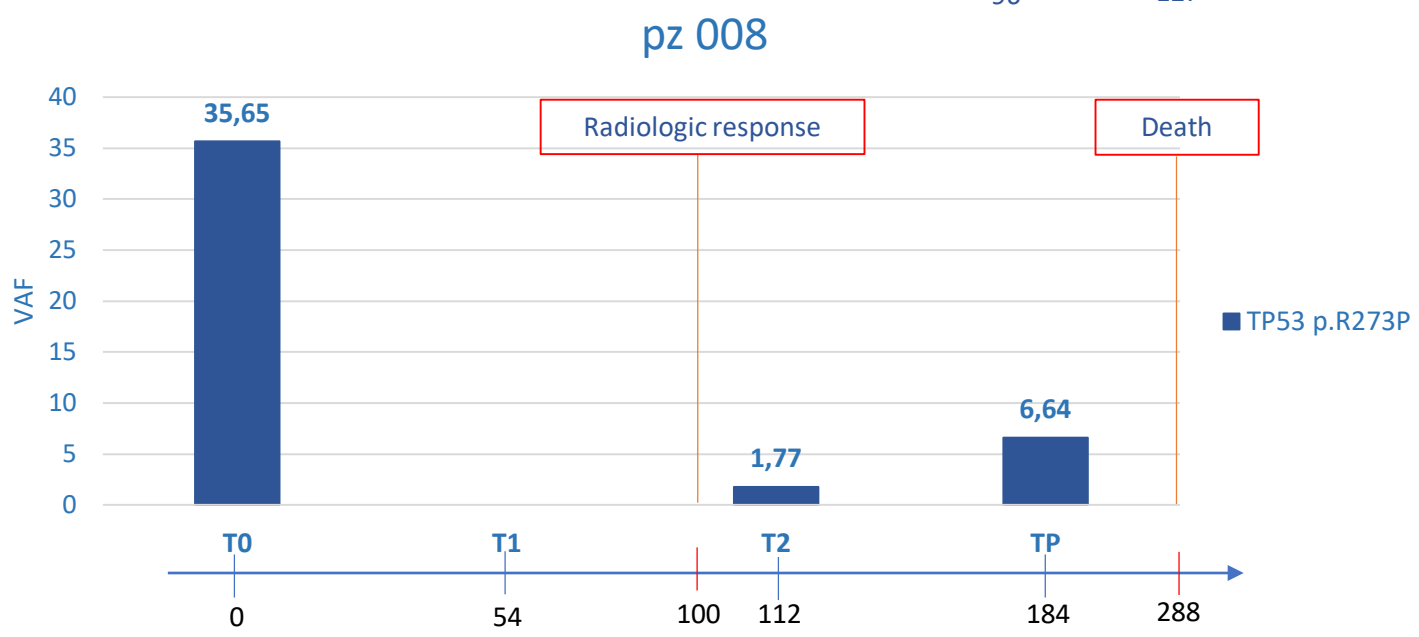
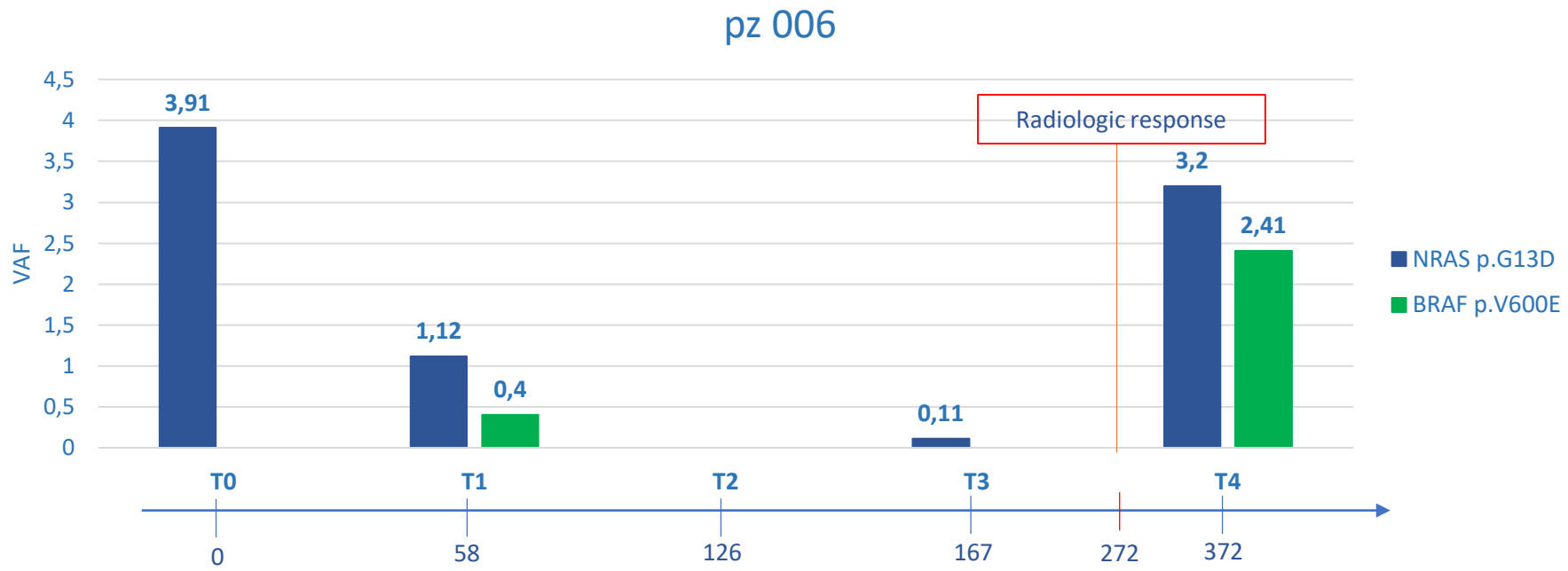


Unpublished data Calabri et al.

Results

ctDNA – longitudinal monitoring

(3)



* Variant Allele Frequency (VAF) was used to quantify each single mutation found in a sample

Unpublished data Calabri et al.

Conclusions

- Liquid biopsy is non-invasive and painless, repeatable multiple times; ctDNA reflects the heterogeneity of the tumor, considered as «real time» cancer biomarker

- Our preliminary results are in line with previously published data on the use of ctDNA in NSCLC for immunotherapy monitoring

- Both variation in the the total content of ctDNA and the type of mutation found could impact on the clinical outcome

- ctDNA may serve as an on-therapy predictor of response to ICI therapy in addition to standard of care imaging in NSCLC:
  - anticipate radiologic response or progression disease

- Liquid biopsy, and ctDNA in particular, has the potential to expand its use in clinical practice:
  - Complementing other biomarkers in immunotherapy decision-making process and as a therapeutic follow-up testing method

Thank you

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