Poster n°300

Tumour-agnostic plasma assay for circulating tumour DNA predicts outcome in recurrent and/or metastatic squamous cell carcinoma of the head and neck treated with a PD-1 inhibitor

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Background

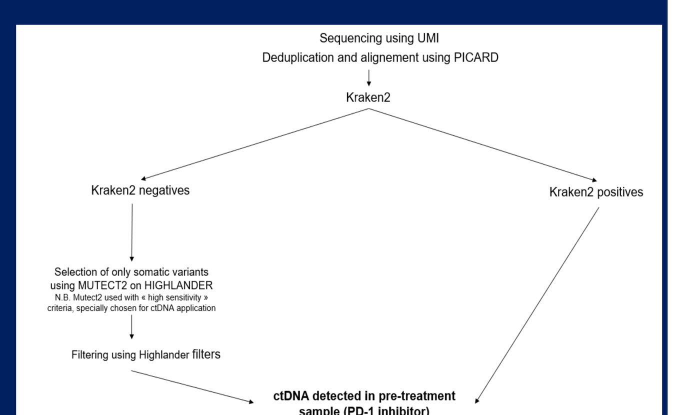
Although PD-1 inhibitors are now standard of care in R/M SCCHN, only around 15-20% of patients will derive long-term benefits from this therapy. Many studies have tried to uncover predictive markers to enable those most likely to respond to be identified early. PDL-1 expression is the only marker currently used in daily clinical practice which selects patients who have a higher probability of response to a PD-1 inhibitor in R/M SCCHN. Other predictive biomarkers, such as the abundance of tumor-infiltrating lymphocytes, tumor mutational burden and specific immune gene signatures, have been investigated; however, they are far from perfect due to significant overlap between responders and non-responders.
ctDNA kinetics are currently being investigated as a biomarker to predict immunotherapy efficacy with the hypothesis that changes in ctDNA quantity could allow early identification of patients likely to progress rapidly or derive long-term benefits.

We developed a ctDNA tumor-agnostic assay with the aim of predicting the efficacy of PD1 inhibitor monotherapy in R/M SCCHN.

Methodology

We developed a tumor-agnostic assay included 37 genes frequently mutated in R/M SCCHN and two HPV16 genes.

NGS was performed after capture using a custom-made in-house panel of 37 genes plus two HPV16 oncogenes (E6 and E7) designed by Twist Bioscience®, with an expected mean coverage of 2000x. The 37 genes were selected because they are frequently mutated in HPVnegative R/M SCCHN. All pre-treatment plasma samples were first analysed with Kraken2 (v. 2.1.2, database "Standard", March 2023), a taxonomic sequence classifier that assigns taxonomic labels to DNA sequences. A patient was considered HPV16-positive if at least one read was assigned to the HPV16 genome in the pre-treatment plasma sample, according to Kraken2. Otherwise, the patient was considered HPV16-negative.



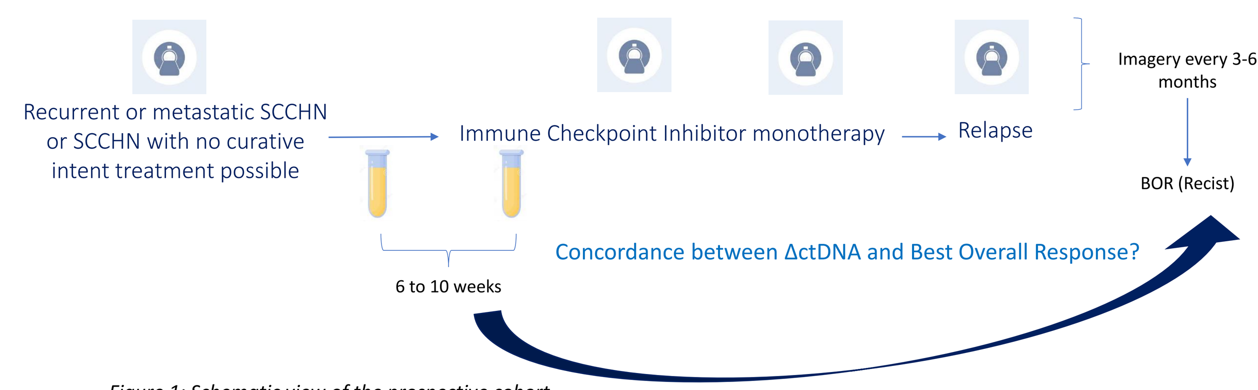
Sequencing using UMI
Deduplication and alignement using PICARD
Kraken2
Kraken2 negatives
Kraken2 positives
Selection of only somatic variants using MUTECT2 on HIGHLANDER
N.B. Mutect2 used with « high sensitivity » criteria, specially chosen for ctDNA application
Filtering using Highlander filters
ctDNA detected in pre-treatment sample (PD-1 inhibitor)

Figure 2: Bio-informatic work-flow

The primary endpoint was the concordance between ctDNA kinetics (ΔctDNA) and best overall response (BOR) according to Response Evaluation Criteria in Solid Tumors version1.1 (RECISTv1.1). ΔctDNA was defined as the difference in mean variant allele frequency (VAF) between the on-treatment sample harvested 6-10 weeks (FU1) after PD-1 inhibitor initiation and the pre-treatment plasma sample (ΔctDNA = mean FU1 VAF - mean pre-treatment VAF).

	Overall
	(N=44)
Sex	
Male	33 (75.0%)
Female	11 (25.0%)
Age (years)	
Median [Min,Max]	68.5 [44.0, 79.0]
Primary location	
Oral cavity	13 (29.5%)
Oropharynx	17 (38.6%)
P16 status	
Negative	10 (22.7%)
Positive	7 (15.9%)
HPV-16 ISH	
Negative	4 (9.0%)
Positive	2 (4.5%)
Missing /not enough material	1 (2.3%)
Hypopharynx	4 (9.0%)
Larynx	3 (6.8%)
Head and Neck unknown primary	6 (13.6%)
Two locations (oropharynx and oral cavity)	1 (2.3%)
CPS	
<1	7 (15.9%)
1-19	16 (36.4%)
≥20	19 (43.2%)
Not enough material	2 (4.5%)

Table 1: Clinical characteristics



Recurrent or metastatic SCCHN or SCCHN with no curative intent treatment possible
Immune Checkpoint Inhibitor monotherapy
Relapse
Imagery every 3-6 months
BOR (Recist)
6 to 10 weeks
Concordance between ΔctDNA and Best Overall Response?

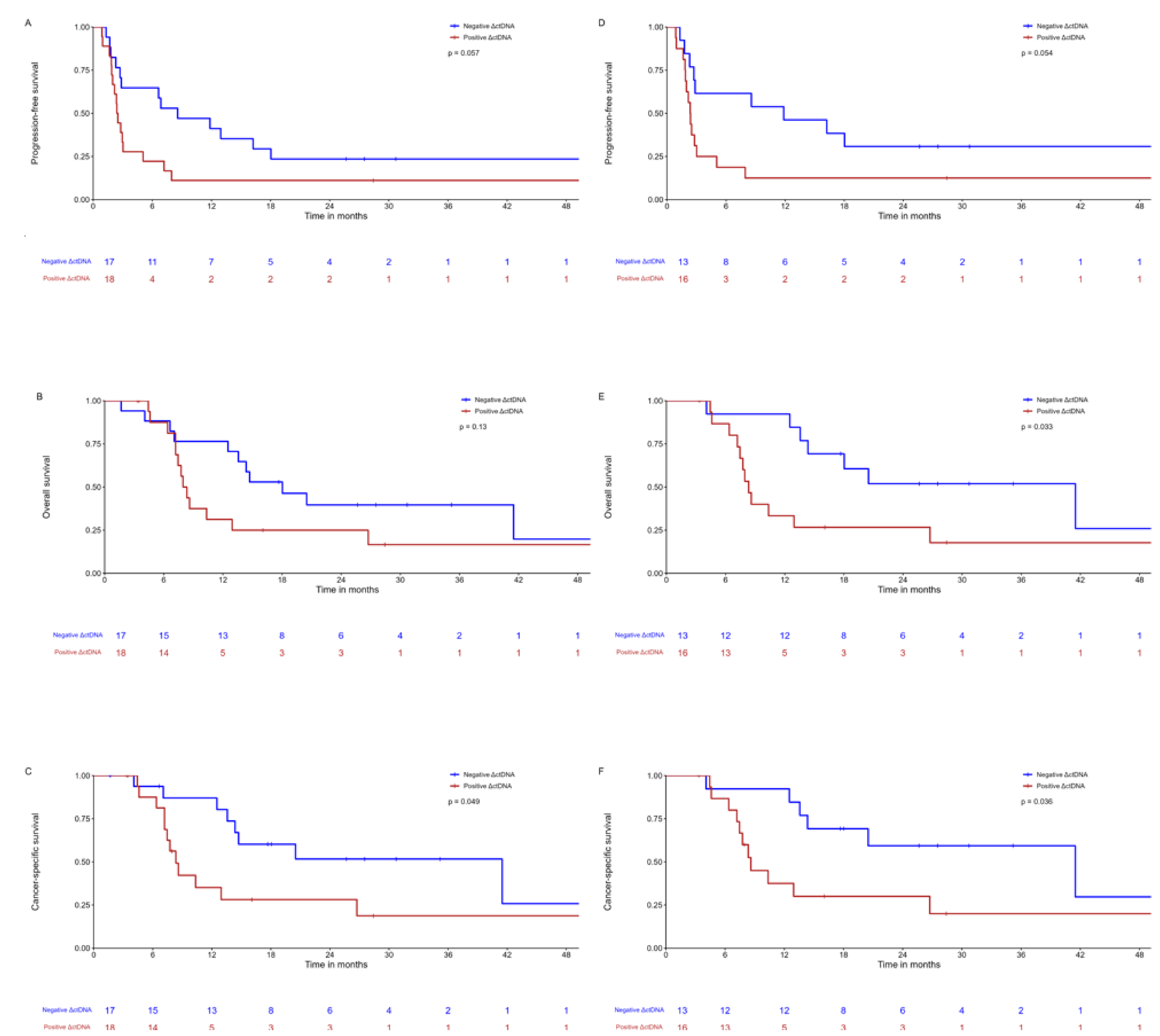
Figure 1: Schematic view of the prospective cohort

Results

Forty-four patients were included, and clinical characteristics are depicted in Table 1.
ctDNA was detected in 35/44 (79%) in the pre-treatment plasma samples.
Among the 35 patients with ctDNA in their pre-treatment samples, 17 and 18 patients had a decrease (negative ΔctDNA) and an increase (positive ΔctDNA) in ctDNA mean VAF, respectively. ctDNA was no longer detectable in seven patients (6 HPV-negative and 1 HPV-positive SCCHN) in the first on-treatment plasma harvested within 6-10 weeks from treatment initiation (FU1).
In the overall cohort, the overall objective response rate (ORR) was 25.7% (9/35) (complete response (CR): 3/35 (8.6%); partial response (PR): 6/35 (17.1%)). Stable disease (SD) and progressive disease (PD) as BOR were achieved in 9 (25.7%) and 17 (48.6%) patients, respectively. Among these 35 patients, no patients had pseudo-progression, defined as initial PD followed by partial or complete response.
Among the 17 patients with negative ΔctDNA, 13 (76.4%) achieved either a CR, PR or SD and 4 (23.5%) had PD. Thirteen (72%) out of 18 with a positive ΔctDNA had PD as BOR (Table 2).

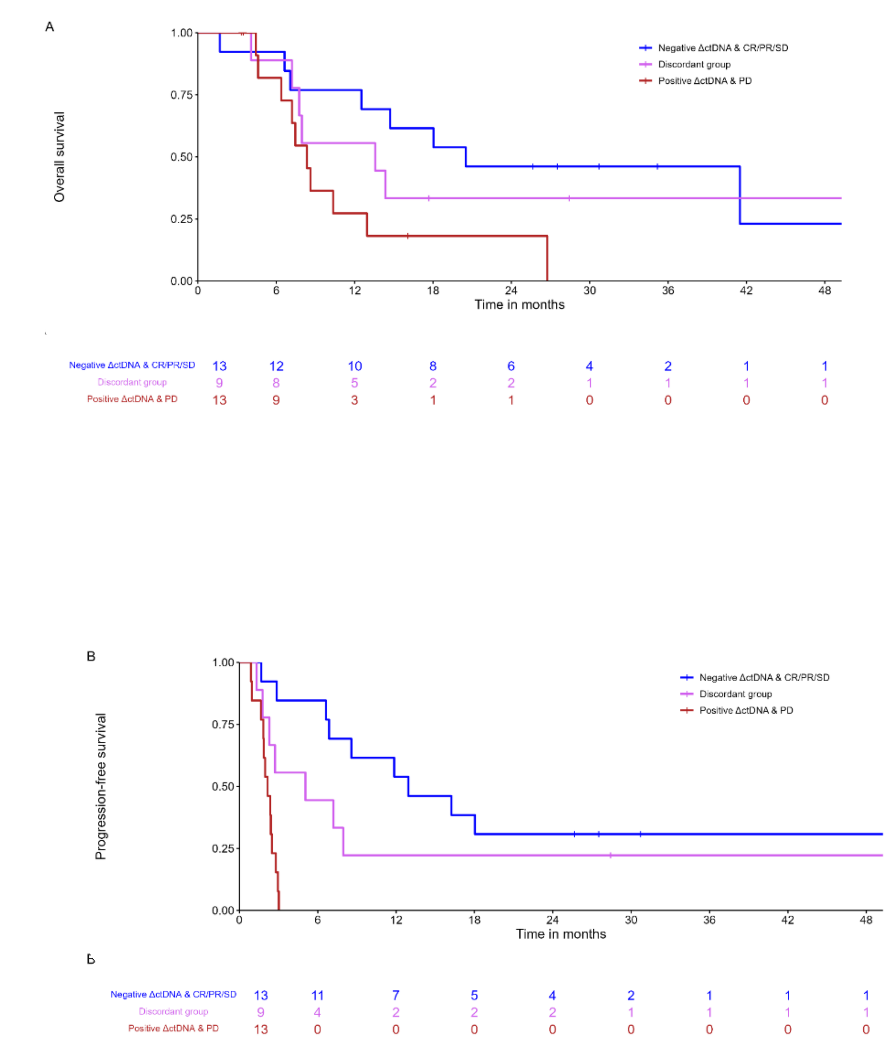
RECISTv1.1	Negative ΔctDNA	Positive ΔctDNA	Total
CR	2 (5.7%)	1 (2.9%)	3 (8.6%)
PR	5 (14.3%)	1 (2.9%)	6 (17.1%)
SD	6 (17.1%)	3 (8.6%)	9 (25.7%)
PD	4 (11.4%)	13 (37.1%)	17 (48.6%)
Total	17 (48.6%)	18 (51.4%)	35 (100%)

Table 2. CR: complete response, PR: partial response, SD: stable disease, PD: progressive disease, RECISTv1.1: Response Evaluation Criteria in Solid Tumors version 1.1
ΔctDNA correctly predicted the BOR according to RECISTv1.1 in 26 (74%) out of 35 patients, whereas 9 (26%) patients had imaging and ΔctDNA discordant results (X² = 4.51; p=0.033).



A
B
C
D
E
F
Progression-free survival
Overall survival
Cancer-specific survival
Time in months
Negative ΔctDNA
Positive ΔctDNA
p = 0.057
p = 0.13
p = 0.049
p = 0.054
p = 0.033
p = 0.036

Figure 3: Kaplan-Meier estimate of progression-free survival (Panels A), overall survival (Panels B), and cancer specific survival (Panels C) for positive and negative ΔctDNA patients in the whole population. Kaplan-Meier estimate of progression-free survival (Panels D), overall survival (Panels E), and cancer specific survival (Panels F) for positive and negative ΔctDNA patients in PD-L1 CPS ≥ 1 population. Blue curves = negative ΔctDNA patients; red curves = positive ΔctDNA patients.



A
B
Overall survival
Progression-free survival
Time in months
Negative ΔctDNA & CR/PR/SD
Discordant group
Positive ΔctDNA & PD

Figure 4: Kaplan-Meier estimate of overall survival (A) and progression-free survival (B) for negative ΔctDNA & CR, PR and SD patients, positive ΔctDNA & PD patients, and discordant patients. Blue curves = negative ΔctDNA negative & CR, PR and SD; red curves = positive ΔctDNA patients & PD; pink curves = discordant patients.

Results

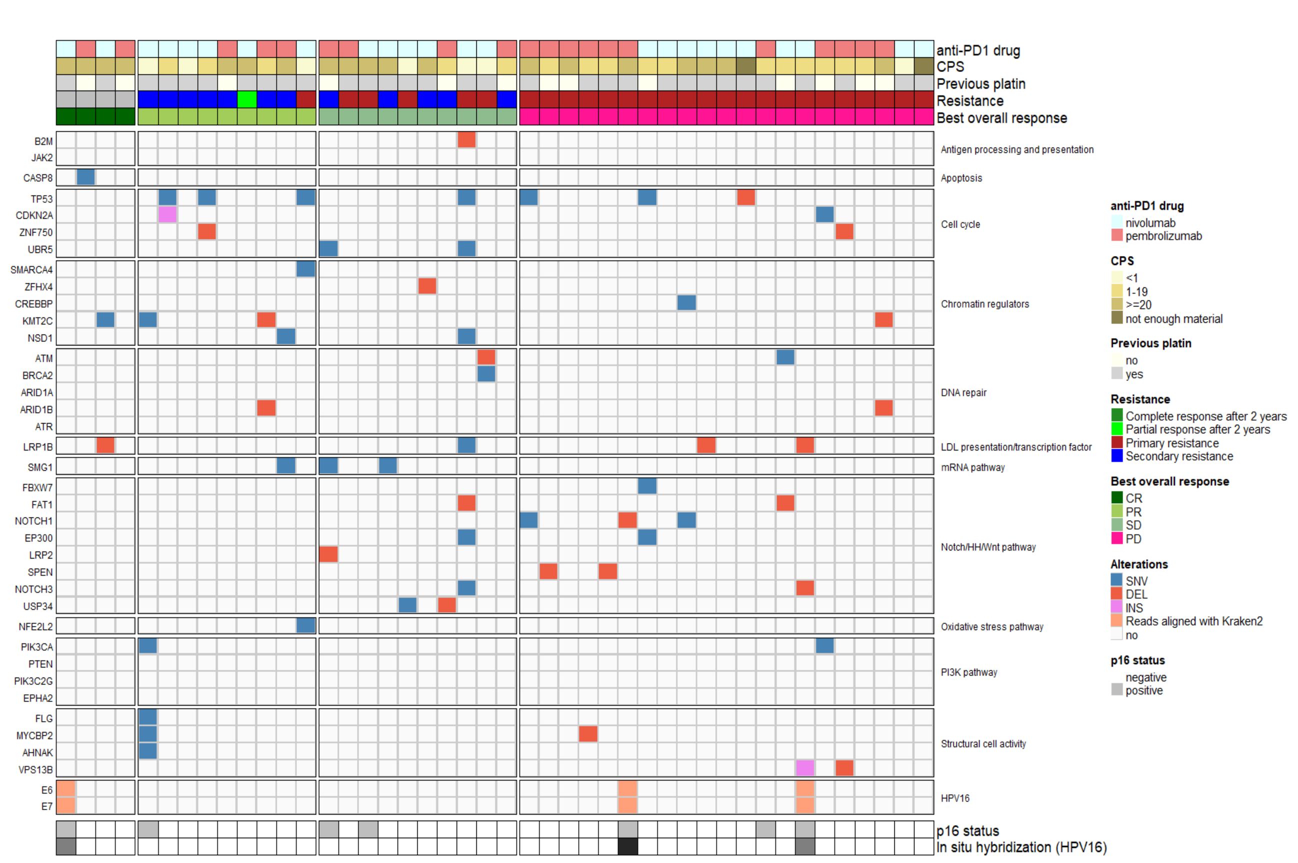
ΔctDNA to predict PFS, OS, and CSS

Median PFS was 8.6 [95% CI: 2.73-18.1] months in the negative ΔctDNA group and 2.5 [95% CI: 2.0-3.0] months in the positive ΔctDNA group (p=0.057, Figure 3A). Median OS was 18.1 [95% CI 12.5-41.5] and 8.2 [95% CI 7.2-13.0] months in the negative and positive ΔctDNA groups, respectively (p=0.13) (Figure 3B). As six patients presumably died from causes other than their cancer (2 from pneumonia, 1 from hip fracture, 1 from sudden death, 1 from Covid infection, and 1 from cerebral vascular accident), we calculated the Cancer-Specific Survival (CSS). CSS was significantly better in the negative ΔctDNA group than in the positive ΔctDNA group: median CSS 41.5 [95% CI: 14.4-NE] and 8.35 [95% CI: 7.2-26.8] months, respectively (p=0.049) (Figure 3C).

ΔctDNA predictive value in different subgroups

In patients with PD-L1 expressing SCCHN (CPS ≥1), there were significant differences between patients with positive and negative ΔctDNA for OS (median OS: 8.4 [95% CI: 7.5-13.0] and 41.5 [95% CI: 14.4-NE] months; p=0.033), and CSS (median OS: 8.6 [95% CI: 7.526.8] months and 41.5 [95% CI: 14.4-NE]; p=0.036), but not PFS (median PFS: 2.4 [95% CI: 1.9-3.03] and 11.9 [95% CI: 2.7-NE] months; p=0.054), (Figures 3D-F).

Some patients had discordant results between ΔctDNA and imaging response. Four patients had a negative ΔctDNA with disease progression whereas five patients had a positive ΔctDNA with either a CR, PR or SD at first imaging evaluation. Interestingly, we observed that these discordant patients (n=9) had an intermediate prognosis. For the discordant group, median OS was 13.6 [95% CI: 7.8-NE] months compared with 20.5 [95% CI: 15.5-NE] months for the patients with negative ΔctDNA and either a CR, PR or SD (p=0.5), and 8.4 [95% CI: 6.4-13] months for the patients with positive ΔctDNA and PD (p=0.2). Similarly, median PFS of the discordant group was 5.1 [95% CI: 2.3-8.0] months compared with 13.0 [95% CI: 6.9-NE] (p=0.2) and 2.2 [95% CI: 1.8-2.5] months (p<0.001), respectively (Figure 4).



anti-PD1 drug
CPS
Previous platin
Resistance
Best overall response
B2M
JAK2
CASP8
TP53
CDKN2A
ZNF750
UBR5
SMARCA4
ZFHX4
CREBBP
KMT2C
NSD1
ATM
BRCA2
ARID1A
ARID1B
ATR
LRP1B
SMG1
FBXW7
FAT1
NOTCH1
EP300
LRP2
SPEN
NOTCH3
USP34
NFE2L2
PIK3CA
PTEN
PIK3C2G
EPHA2
FLG
MYCBP2
AHNAK
VPS13B
E6
E7
Antigen processing and presentation
Apoptosis
Cell cycle
Chromatin regulators
DNA repair
LDL presentation/transcription factor
mRNA pathway
Notch/HH/Wnt pathway
Oxidative stress pathway
PI3K pathway
Structural cell activity
HPV16
p16 status
In situ hybridization (HPV16)
anti-PD1 drug
nivolumab
pembrolizumab
CPS
<1
1-19
>=20
not enough material
Previous platin
no
yes
Resistance
Complete response after 2 years
Partial response after 2 years
Primary resistance
Secondary resistance
Best overall response
CR
PR
SD
PD
Alterations
SNV
DEL
INS
Reads aligned with Kraken2
no
p16 status
negative
positive
In situ hybridization (HPV16)
negative
positive
unknown

Figure 5: Mutations in pre-treatment plasma sample and patient characteristics. PD1: Programmed cell death 1, CPS: combined positive score, CR: complete response, PR: partial response, SD: stable disease, PD: progressive disease, SNV: single nucleotide variation, DEL: deletion, INS: insertion

Results

The mutations considered to be damaging and found in the pre-treatment plasma samples are depicted in Figure 5. Interestingly, we observed that inactivating mutations in genes implicated in the Notch/Hedgehog/Wnt pathways were only identified in patients with SD or PD as BOR and not in patients who had a CR or PR. There was a significant difference in PFS between patients with pre-treatment plasma harboring mutations in genes implicated in the Notch/Hedgehog/Wnt pathways compared to the others: median 2.07 [95%IC: 1.8-2.7] vs 6.8 [95%IC: 2.9-13.0] months (p=0.024). There was, however, no statistical difference in OS: 8.2 [95%IC: 6.6-13.6] vs 14.7 [95% IC: 8.6-41.5] months (p=0.083).

Another patient with primary resistance had a pre-treatment ctDNA *B2M* mutation. In contrast, in patients with long-term CR, we observed one patient with *CASP8* (p.E123Q) mutation and another with *KMT2C* (p.C1150Y) and *LRP1B* (p.G683fs) mutations.

Conclusions

Tumor-agnostic ctDNA analysis for HPV-negative and HPV-positive R/M SCCHN is feasible. ctDNA dynamics show promising results in predicting response to immunotherapy in R/M SCCHN. Further investigations are necessary to integrate ctDNA kinetics with other prognostic and predictive biomarkers to better predict treatment and cancer outcomes.