Letter to the editor

Early relapse detection by monitoring of circulating cell-free DNA in patients with localized head and neck squamous cell carcinoma: A subgroup analysis of the multicenter randomized clinical trial IMSTAR-HN

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Head and Neck Squamous Cell Carcinoma (HNSCC) belongs to the top ten most common malignancies worldwide [1,2]. Treatment of loco-regional disease includes tumor surgery and neck dissection followed by (chemo)radiotherapy in cases with moderate to high risk [2,3]. The relapse rate in such cases is high with approximately 50% of patients developing relapse within 2 years of treatment [4,5]. To reduce relapse rates, a number of trials have set out to optimize adjuvant systemic treatment, but none of these approaches has resulted in a modification of the cisplatinum-based standard of care so far [6,7]. Lately, the efficacy of immune checkpoint blockade in relapsed/refractory HNSCC has inspired trial concepts evaluating the addition of PD-1/PD-L1/CTLA-4

Table 1
Baseline characteristics of patients from the IMSTAR-HN liquid biopsy cohort.

Characteristics	IMSTAR liquid biopsy subcohort n = 19
Sex, No. (%)	
Male	14 (74%)
Female	5 (26%)
Age	
Median years (range)	65 (32–76)
ECOG performance status, No. (%)	
0	12 (63 %)
1	7 (37 %)
Site of primary tumor, No. (%)	2
Oral cavity	(10.5%)
Oropharynx	4 (21%)
Hypopharynx	7 (37%)6
Larynx	(31.5%)
Tumor Grade	
G2	12 (63%)
G2/G3	4 (21%)
G3	3 (16%)

ECOG, Eastern Cooperative Oncology Group.

targeting antibodies in the adjuvant treatment setting to prevent or delay relapse [8,9]. Yet, in addition to these ongoing efforts, early relapse detection will need to become a priority since many HNSCC relapses are again loco-regional [10] and – if detected early – can still be cured [11,12].

In this line of reasoning, non-invasive blood-based biomarkers that are surrogates for residual tumor cells and may predict imminent relapse may be very helpful to guide the intensity of clinical and radiological surveillance. The measurement of tumor-derived cell-free DNA (cfDNA) – often referred to as liquid biopsy – is minimally invasive, cost and time efficient and can be performed as often as necessary [13,14]. Previous trials in different tumor entities have shown that for detection of locally confined tumors or minimal residual disease after surgery, very sensitive liquid biopsy techniques are required [15–18]. Digital droplet PCR (ddPCR) is one such method with a sensitivity down to variant allele frequencies of 0.01%, however, in contrast to next-generation sequencing (NGS) usually providing information on only one mutation at a time.

In the work presented here, we performed serial liquid biopsy monitoring by ddPCR in a subsample of 19 patients from the IMSTAR-HN trial (Table 1) to identify HNSCC patients at risk for relapse after treatment with curative intent. The IMSTAR-HN study evaluated the safety and tolerability of an immune checkpoint inhibitor regimen added to the standard of (chemo)radiation in the (neo)adjuvant treatment of patients with a new diagnosis of HNSCC [9]. Twelve patients were treated in the immunotherapy arm of the trial, while seven patients received standard of care. We used targeted NGS to determine mutation the individual tumor mutation profiles in all patients followed by design of mutation-specific ddPCR assays as described in the Detailed Methods Section provided as Supplemental Data. On average, eight prospectively collected plasma samples were analyzed for the presence of circulating tumor DNA (ctDNA) over treatment and follow-up and results were correlated with clinical endpoints.

Fig. 1 shows the mutational distribution in our cohort. In 18 out of 19 cases, at least one mutation was detected. As expected, the by far most
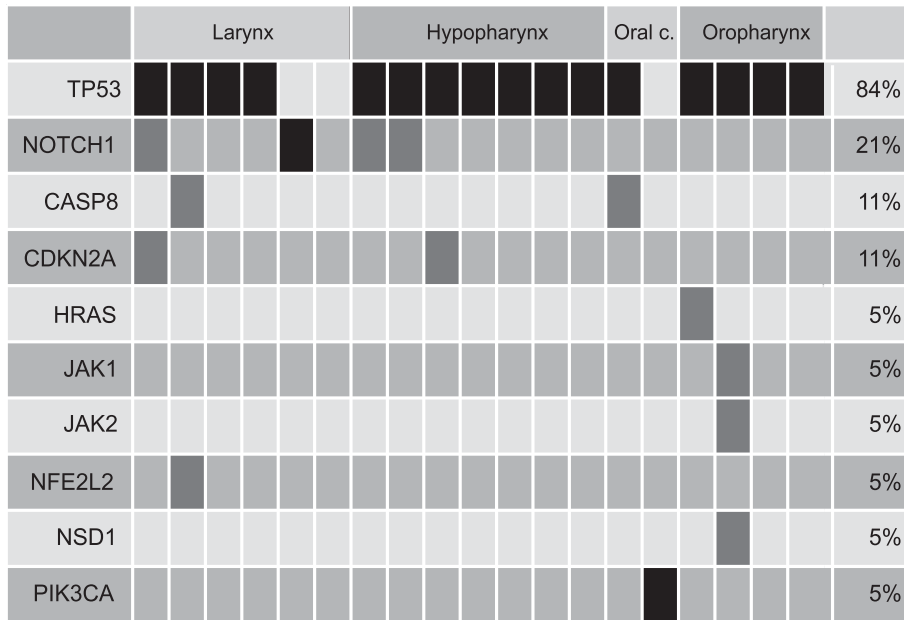
	Larynx	Hypopharynx	Oral c.	Oropharynx	
TP53					84%
NOTCH1					21%
CASP8					11%
CDKN2A					11%
HRAS					5%
JAK1					5%
JAK2					5%
NFE2L2					5%
NSD1					5%
PIK3CA					5%

Fig. 1. Overview of tumor mutations in the IMSTAR-HN liquid biopsy cohort. Oncoprint of all mutations that were found in FFPE samples from baseline biopsy or surgical removal. *Oral c., Oral cavity.*

frequently mutated gene that showed aberrations in more than 80% of cases was TP53. For 17 out of 18 patients with characterized mutations, individual probes for ddPCR could be successfully designed and validated on individually synthesized gBlocks. Thresholds were set and later applied to liquid biopsy analysis. All ddPCR assays were subsequently performed on tumor tissue DNA to validate the mutations found by NGS. Fig. 2 shows serial liquid biopsy monitorings for each of the evaluable patients. Eleven patients were liquid biopsy positive before treatment initiation. Upon treatment, eight of these eleven patients fully cleared their ctDNA after surgery as defined by two consecutive negative measurements. None of the patients with full ctDNA clearance showed disease recurrence. A total of four patients showed newly emerging or persistent ctDNA positivity at a minimum of two consecutive time points in the treatment course. With a median follow-up of 93 weeks, two out of these four cases had disease progression. One of them showed a local failure at 73 weeks from study enrolment (pt-18). This patient had never been liquid biopsy negative on two consecutive measurements. The other patient had a distant failure with widespread metastases 36 weeks from study enrolment (pt-14). The time between first ctDNA detection and clinical/radiological recurrence was 18 weeks in pt-14. Two individuals (pt-2 and pt-15) with persistence of ctDNA positivity in the course of follow-up did not develop relapse during the observation period (Fig. 3). Both cases showed low level undulating variant allele frequencies over time. Due to this ctDNA profile, we hypothesized that liquid biopsy positivity indicates minimal residual cancer that is partially controlled and does - at least until the last data cut-off - not result in manifest relapse. Interestingly, both cases had received immunotherapy in the neoadjuvant treatment setting and pt-15 also in the adjuvant setting which could be hypothesized to account for long-term minimal residual disease control reflected by the ctDNA dynamics in these patients.

Taken together, our data shows that liquid biopsy disease monitoring using tumor-specific ddPCR assays is feasible in localized HNSCC. It suggests that persistent or newly emerging ctDNA has the potential to identify patients at risk for disease recurrence after treatment with curative intent. Thereby, it adds to a still rather small body of evidence on ddPCR-based liquid biopsies in HNSCC as personalized tool for sensitive disease monitoring [19–22].
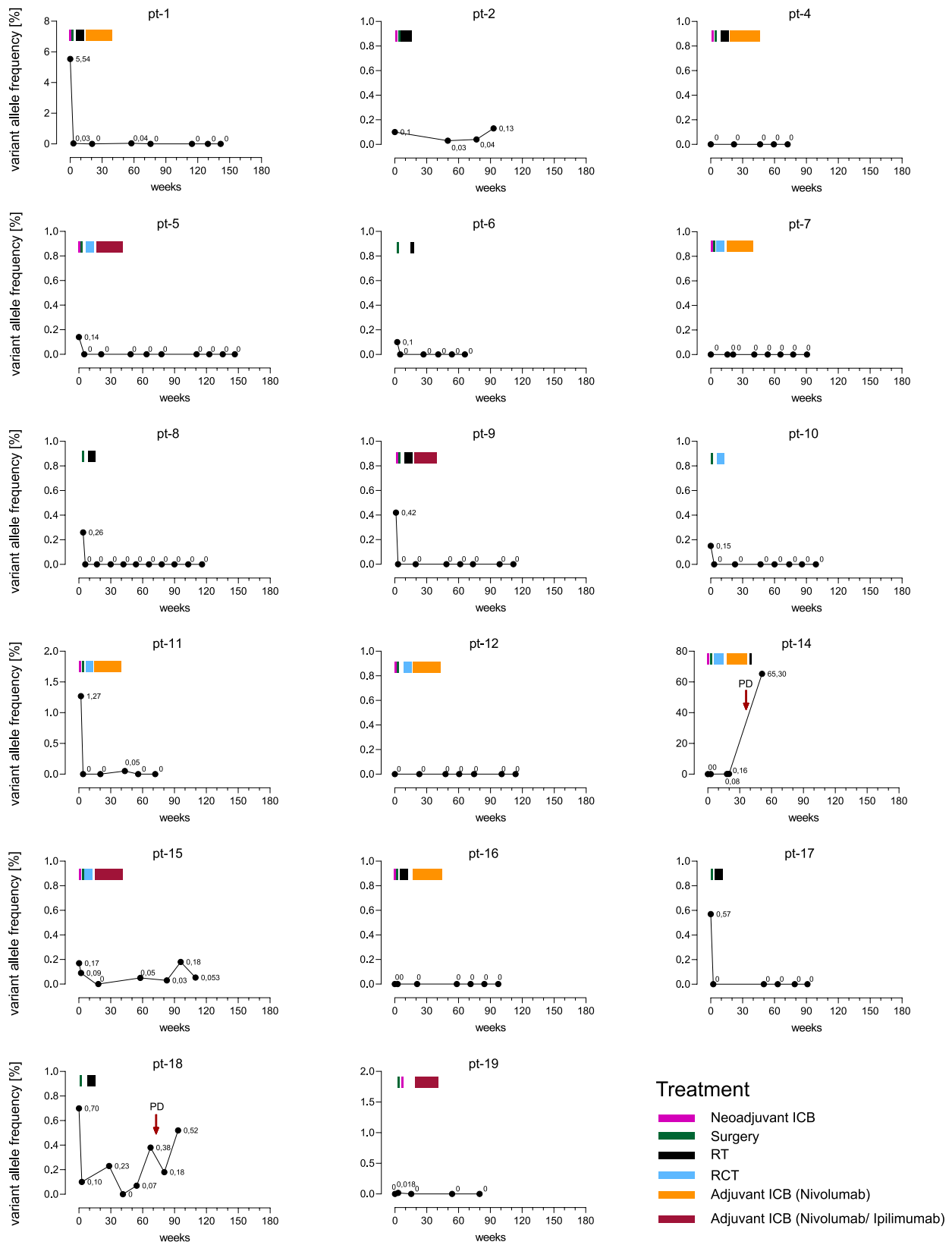
Fig. 2. Longitudinal disease monitoring in serial plasma samples of IMSTAR-HN liquid biopsy cohort. The monitoring was performed by ddPCR using probes specific for one of the driver mutations identified by NGS on tumor tissue. *ICB, Immune Checkpoint Blockade; PD, Progressive Disease; RCT, Radio-chemo-therapy; RT, Radiotherapy.*

Declaration of Competing Interest

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.oraloncology.2022.105733.

References

[1] Alsahafi E, Begg K, Amelio I, et al. Clinical update on head and neck cancer: molecular biology and ongoing challenges. Cell Death Dis 2019;10:540.
[2] Chow LQM. Head and Neck Cancer. N Engl J Med 2020;382:60–72.
[3] Bernier J, Domenge C, Ozsahin M, et al. Postoperative irradiation with or without concomitant chemotherapy for locally advanced head and neck cancer. N Engl J Med 2004;350:1945–52.
[4] Rettig EM, D'Souza G. Epidemiology of head and neck cancer. Surg Oncol Clin N Am 2015;24:379–96.
[5] Argiris A, Karamouzis MV, Raben D, et al. Head and neck cancer. Lancet 2008;371: 1695–709.
[6] Kiyota N, Tahara M, Fujii H, et al. Phase II/III trial of post-operative chemo-radiotherapy comparing 3-weekly cisplatin with weekly cisplatin in high-risk patients with squamous cell carcinoma of head and neck (JCOG1008). J Clin Oncol 2020;38:6502.
[7] Lacas B, Carmel A, Landais C, et al. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): An update on 107 randomized trials and 19,805 patients, on behalf of MACH-NC Group. Radiother Oncol 2021;156:281–93.
[8] Schoenfeld JD, Hanna GJ, Jo VY, et al. Neoadjuvant Nivolumab or Nivolumab Plus Ipilimumab in Untreated Oral Cavity Squamous Cell Carcinoma: A Phase 2 Open-Label Randomized Clinical Trial. JAMA Oncol 2020;6:1563–70.
[9] Zech HB, Moeckelmann N, Boettcher A, et al. Phase III study of nivolumab alone or combined with ipilimumab as immunotherapy versus standard of care in resectable head and neck squamous cell carcinoma. Future Oncol 2020;16:3035–43.
[10] Leeman JE, Li JG, Pei X, et al. Patterns of Treatment Failure and Postrecurrence Outcomes Among Patients With Locally Advanced Head and Neck Squamous Cell Carcinoma After Chemoradiotherapy Using Modern Radiation Techniques. JAMA Oncol 2017;3:1487–94.
[11] Mehanna H, Wong WL, McConkey CC, et al. PET-CT Surveillance versus Neck Dissection in Advanced Head and Neck Cancer. N Engl J Med 2016;374:1444–54.
[12] Mehanna H, Kong A, Ahmed SK. Recurrent head and neck cancer: United Kingdom National Multidisciplinary Guidelines. J Laryngol Otol 2016;130:S181–90.
[13] Schmidt H, Kulasinghe A, Kenny L, et al. The development of a liquid biopsy for head and neck cancers. Oral Oncol 2016;61:8–11.
[14] Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 6:224ra24 2014.
[15] Garcia J, Kamps-Hughes N, Geiguer F, et al. Sensitivity, specificity, and accuracy of a liquid biopsy approach utilizing molecular amplification pools. Sci Rep 2021;11: 10761.
[16] Alborelli I, Generali D, Jermann P, et al. Cell-free DNA analysis in healthy individuals by next-generation sequencing: a proof of concept and technical validation study. Cell Death Dis 2019;10:534.
[17] Lanman RB, Mortimer SA, Zill OA, et al. Analytical and Clinical Validation of a Digital Sequencing Panel for Quantitative, Highly Accurate Evaluation of Cell-Free Circulating Tumor DNA. PLoS ONE 2015;10:e0140712.
[18] Stetson D, Ahmed A, Xu X, et al. Orthogonal Comparison of Four Plasma NGS Tests With Tumor Suggests Technical Factors are a Major Source of Assay Discordance. JCO Precis Oncol 2019;1–9.
[19] van Ginkel JH, Huibers MMH, van Es RJJ, et al. Droplet digital PCR for detection and quantification of circulating tumor DNA in plasma of head and neck cancer patients. BMC Cancer 2017;17:428.
[20] Rösch R, Kerle I, Pfarr N, et al. Clinical application of personalized ddPCR assays in liquid profiling of blood and saliva allows early recurrence detection of head and neck tumors. Laryngorhinootologie 2021;100.
[21] Fung SYH, Chan KCA, Wong EWY, et al. Droplet digital PCR of tumor suppressor gene methylation in serial oral rinses of patients with head and neck squamous cell carcinoma. Head Neck 2021;43:1812–22.
[22] Hayashi M, Guerrero-Preston R, Sidransky D, et al. Paired box 5 methylation detection by droplet digital PCR for ultra-sensitive deep surgical margins analysis of head and neck squamous cell carcinoma. Cancer Prev Res (Phila) 2015;8: 1017–26.

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