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 [Letter]

Hanna Jonas, Donjete Simnica, Lara Bußmann, Henrike Zech, Johannes Doescher, Simon Laban, Chia-Jung Busch, Mascha Binder

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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

Letter to the Editor

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 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].

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.

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

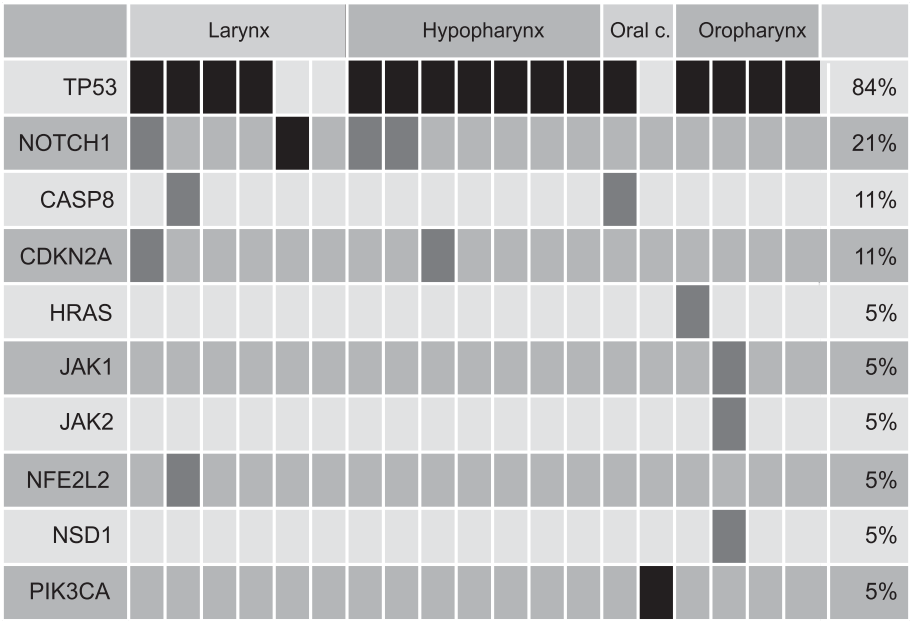

Fig. 1. Overview of tumor mutations in the IMSTAR-HN liquid biopsy cohort. Oncoprint of all mutations that were found in FFPE samples from baseline biopsy or surgical removal. *Oral c., Oral cavity.*

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].

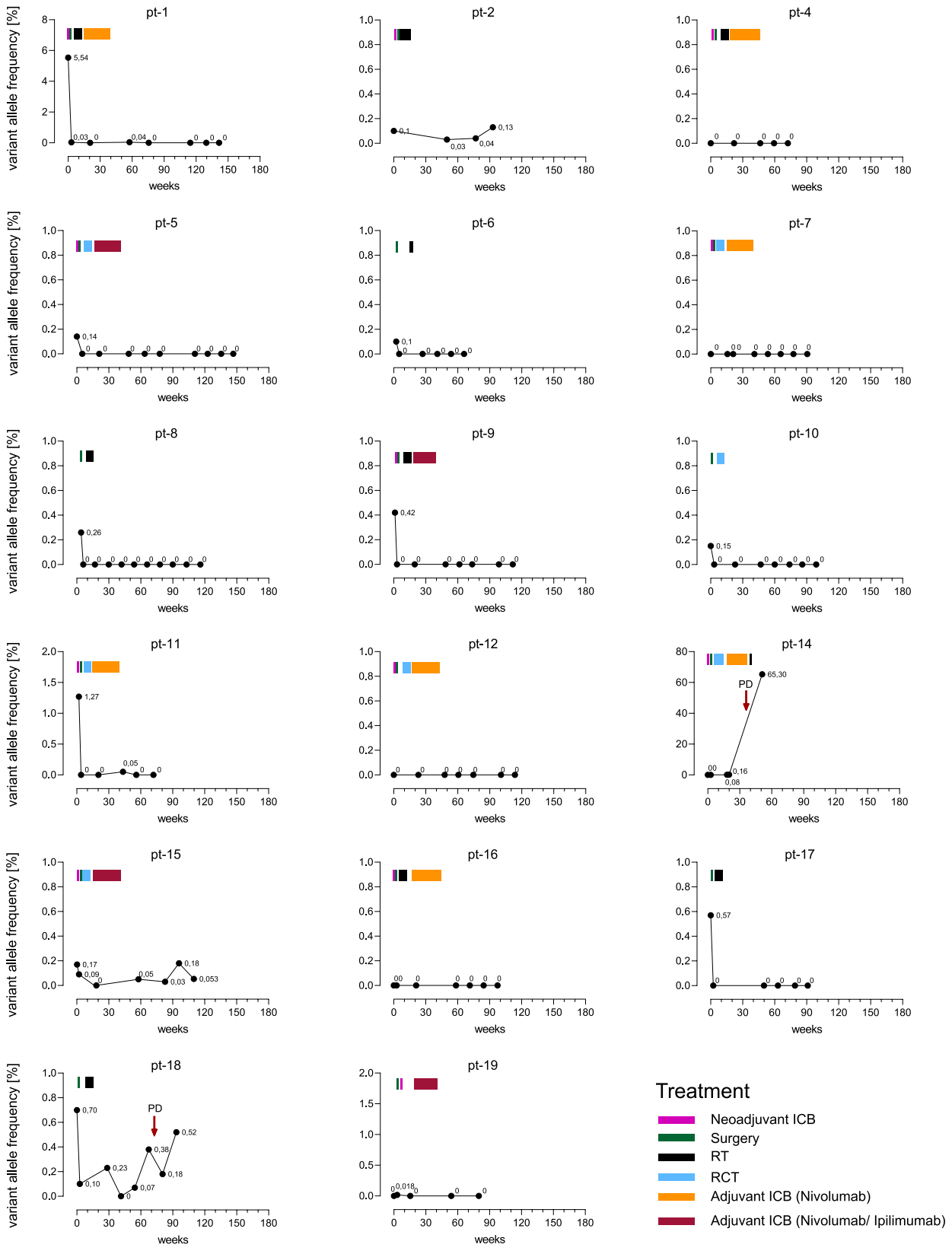

Fig. 2. Longitudinal disease monitoring in serial plasma samples of IMSTAR-HN liquid biopsy cohort. The monitoring was performed by ddPCR using probes specific for one of the driver mutations identified by NGS on tumor tissue. *ICB, Immune Checkpoint Blockade; PD, Progressive Disease; RCT, Radio-chemo-therapy; RT, Radiotherapy.*

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data and materials availability

The project was funded by a translational research fund provided by BMS (to CJB and MB).

Appendix A. Supplementary data

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

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Hanna Jonas[1], Donjete Simnica[1]
Department of Internal Medicine IV, University Hospital Halle, Martin-Luther University Halle-Wittenberg, Germany

Lara Bußmann
Department of Otorhinolaryngology, Head and Neck Surgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
Mildred Scheel Cancer Career Center HaTriCS4, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Henrike Zech
Department of Otorhinolaryngology, Head and Neck Surgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Johannes Doescher, Simon Laban
Department of Otorhinolaryngology, Head and Neck Surgery, University Medical Center Ulm, Germany

Chia-Jung Busch[*]
Department of Otorhinolaryngology, Head and Neck Surgery, University Medicine Greifswald, Germany
Department of Otorhinolaryngology, Head and Neck Surgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Mascha Binder[**]
Department of Internal Medicine IV, University Hospital Halle, Martin-Luther University Halle-Wittenberg, Germany

[*] Corresponding author at: Department of Otorhinolaryngology, Head and Neck Surgery, University Medicine Greifswald, Ferdinand-Sauerbruch-Str., 17475 Greifswald, Germany.

[**] Corresponding author at: Department of Internal Medicine IV, University Hospital Halle, Martin-Luther University Halle-Wittenberg, Ernst-Grube-Str. 40, 06120 Halle (Saale), Germany.
E-mail address: chia-jung.busch@med.uni-greifswald.de (C.-J. Busch).
E-mail address: Mascha.Binder@uk-halle.de (M. Binder).

[1] These first and senior authors contributed equally.