J Cancer Immunol. 2024;6(3):116-118.

Journal of Cancer Immunology

Commentary

# Next Generation Sequencing (NGS) of Matched Tissue and Circulating DNA (ctDNA) in Patients with Squamous Cell Carcinoma of the Head and Neck (SCCHN)

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**Received date:** July 19, 2024, **Accepted date:** August 17, 2024

**Citation:** Economopoulou P, Kotsantis I, Psyrri A. Next Generation Sequencing (NGS) of Matched Tissue and Circulating DNA (ctDNA) in Patients with Squamous Cell Carcinoma of the Head and Neck (SCCHN). J Cancer Immunol. 2024;6(3):116-118.



## Keywords

Head and neck, Metastases, Squamous cell carcinoma

## Commentary

Despite advances in multimodality treatment, a significant proportion of patients with squamous cell carcinoma of the head and neck (SCCHN) eventually presents with either local recurrence or distant metastases. Although imaging and tissue biopsy are the cornerstones for the diagnosis of relapse, tissue acquisition is impeded by the complex anatomy of head and neck tumors which frequently necessitates open biopsies via examination under anesthesia; therefore, understanding the underlying molecular biology is challenging. In this context, liquid biopsy, a general term which encompasses the analysis of cancer-related signals in biological fluids such as blood and saliva, enables a comprehensive real-time view of cancer progression [1]. Liquid biopsy has been introduced in 2010 [2] and mainly includes the analysis of circulating tumor cells (CTCs) or circulating tumor DNA (ctDNA) that represent tumor-derived circulating biomarkers shed into the blood following necrosis, apoptosis or active secretion [3].

Clinical applications of liquid biopsy vary from early detection of cancer [4,5], and identification of patients with localized cancer at high risk of relapse [6] to real-time monitoring of treatment efficacy. Most importantly, ctDNA detected in blood plasma has been demonstrated to reflect the mutational profile of the primary tumor at the specific time of the blood sampling. This “tracking” of tumor evolution would require repeated tissue biopsies, which are invasive procedures associated with complications [7]. In addition, as reported by Gerlinger *et al.*, there is significant genetic heterogeneity between primary tumor and different metastatic sites depending on the location [8].

Based on these considerations, our group conducted a pilot study that sought to assess the concordance of somatic mutations in matched tissue and ctDNA samples obtained from patients with localized SCCHN and evaluate the correlation of changes in ctDNA levels at three timepoints with survival [9]. Blood samples were collected at baseline, at the end of treatment and at disease progression. The molecular analysis of tissue and blood samples was performed by a next-generation sequencing (NGS) UID error correction-based technology (SafeSeq) that was used to detect a restricted group of mutations in four selected genes (TP53, CDKN2A, HRAS and PI3KCA). Indeed, the complex process of carcinogenesis results in a plethora of genomic aberrations in solid tumors, and this might complicate the sensitivity of ctDNA analysis. Due to the unknown “in-depth” genetic profile of the tumor, the ctDNA assay should at least incorporate the most frequent genomic alterations in possible cancer genes. In our study, the selection of genes was based on published data regarding genotyping of SCCHN from the Cancer Genome Atlas (TCGA) [10]; TP53, CDKN2A, HRAS and PI3KCA are fundamental genes in head and neck carcinogenesis and known to yield abundant patient coverage of the SCHHN mutational landscape defined in TCGA [10].

We consider our study a pioneering effort in the field of liquid biopsy in head and neck cancer for a multitude of reasons. First, it is a prospective study evaluating serial plasma samples obtained at specific timepoints (baseline, end of treatment, disease progression) in patients with locally advanced SCCHN treated with chemoradiotherapy with curative intent. We demonstrate that the detection of genomic alterations in plasma ctDNA is feasible using a highly sensitive NGS-based method. In our cohort, 41% of patients were found to be ctDNA positive at baseline [9]. This percentage has been found to be much higher in other studies, where different principles for detection are used [11,12]. Briefly, ctDNA technologies can be subcategorized into targeted approaches that seek to identify mutations in a set of predefined genes and untargeted approaches, such as whole-genome sequencing (WES) that seek to assess the whole genome [13]. For example, the RaDaR assay is a so-called "tumor-informed" assay that is based upon personalized multiplex PCR amplification of cell free DNA following identification of tumor-specific variants by WES of the primary tumor [12].

Second, we show that the concordance of genotyping results between ctDNA at baseline and tumor DNA is low (53.3% in our cohort [9] and widely ranging between 2.7% and 66% in other studies [14-17]), possibly mirroring the greater tumor heterogeneity of SCCHN. Indeed, the molecular signatures of HPV-associated SCCHN are strikingly different from non-HPV-related (HPV-negative) SCCHN [10]. In addition, SCCHNs are classified in four molecular subgroups, each with distinct genetic profile [10]. In a recent study that included more than 3,000 patients with non-small cell lung, colorectal, breast and prostate cancer, patients underwent concurrent tissue and ctDNA NGS [18]. It was found that the concordance between tissue and ctDNA-based assays was 66.4%, which was highest in colorectal cancer (75%) and lowest in prostate cancer (51.2%). Although it is widely accepted that tissue-based NGS is the standard method to guide therapeutic options in solid tumors, it might be unable to capture molecular intratumor and intertumor (across different metastatic lesions) heterogeneity and, most importantly, the biological variations of subclones that contribute to drug resistance [19]. On the other hand, ctDNA NGS analysis might provide false positive results attributed to clonal hematopoiesis of indeterminate potential (CHIP) [20]. Thus, concurrent NGS testing might provide additional information to timely guide therapeutic options to each patient with cancer.

Third, we found that the presence of mutations in tissue and plasma was associated with decreased survival [9], which was consistent with one previous report in SCCHN [14]. Similar findings for ctDNA analysis have been reported in metastatic breast cancer and non-small cell lung cancer [21-23]. Indeed, TP53 mutations, which are common in SCCHN are associated with short survival and resistance to therapy. However, change in ctDNA dynamics did not correlate with survival in our study; we consider this a result of the small sample size. In non-small cell lung cancer, lack of decrease in ctDNA levels post immunotherapy or chemo-immunotherapy has been associated with inferior survival. Therefore, a longitudinal ctDNA model could be used as a valuable tool to predict response to treatment much earlier than conventional imaging and is currently being evaluated in multiple clinical trials as a stratification factor to guide modifications in treatment approaches.

In conclusion, we conducted a pilot study which is proof of concept that liquid biopsy in SCCHN enables real-time molecular characterization of tumors and may be correlated with survival. The discovery of ESR1 and PI3KCA mutations as emerging biomarkers that are detected in ctDNA and guide treatment decisions in breast cancer paved the way for more clinical applications in other solid malignancies [24,25]. As the emerging field of ctDNA research has opened new avenues for cancer diagnostics and has offered many opportunities for applications in clinical practice, we eagerly await large prospective studies which will demonstrate the clinical utility of liquid biopsy in SCHHN.

## References

1. Alix-Panabières C, Marchetti D, Lang JE. Liquid biopsy: from concept to clinical application. Sci Rep. 2023 Dec 7;13(1):21685.

2. Pantel K, Alix-Panabières C. Circulating tumour cells in cancer patients: challenges and perspectives. Trends Mol Med. 2010 Sep;16(9):398-406.

3. Stergiopoulou D, Markou A, Strati A, Zavridou M, Tzanikou E, Mastoraki S, et al. Comprehensive liquid biopsy analysis as a tool for the early detection of minimal residual disease in breast cancer. Sci Rep. 2023 Jan 23;13(1):1258.

4. Nicholson BD, Oke J, Virdee PS, Harris DA, O'Doherty C, Park JE, et al. Multi-cancer early detection test in symptomatic patients referred for cancer investigation in England and Wales (SYMPLIFY): a large-scale, observational cohort study. Lancet Oncol. 2023 Jul;24(7):733-43.

5. Lennon AM, Buchanan AH, Kinde I, Warren A, Honushefsky A, Cohain AT, et al. Feasibility of blood testing combined with PET-CT to screen for cancer and guide intervention. Science. 2020 Jul 3;369(6499):eabb9601.

6. Coombes RC, Page K, Salari R, Hastings RK, Armstrong A, Ahmed S, et al. Personalized Detection of Circulating Tumor DNA Antedates Breast Cancer Metastatic Recurrence. Clin Cancer Res. 2019 Jul 15;25(14):4255-63.

7. Alix-Panabières C, Pantel K. Liquid Biopsy: From Discovery to Clinical Application. Cancer Discov. 2021 Apr;11(4):858-73.

8. Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, Endesfelder D, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012 Mar 8;366(10):883-92.

9. Economopoulou P, Spathis A, Kotsantis I, Maratou E, Anastasiou

M, Moutafi MK, et al. Next-generation sequencing (NGS) profiling of matched tumor and circulating tumor DNA (ctDNA) in head and neck squamous cell carcinoma (HNSCC). Oral Oncol. 2023 Apr;139:106358.

10. Cancer Genome Atlas Network. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature. 2015 Jan 29;517(7536):576-82.

11. Flach S, Howarth K, Hackinger S, Pipinikas C, Ellis P, McLay K, et al. Liquid BIOpsy for MiNimal RESidual DiSease Detection in Head and Neck Squamous Cell Carcinoma (LIONESS)-a personalised circulating tumour DNA analysis in head and neck squamous cell carcinoma. Br J Cancer. 2022 May;126(8):1186-95.

12. Sanz-Garcia E, Zou J, Avery L, Spreafico A, Waldron J, Goldstein D, et al. Multimodal detection of molecular residual disease in high-risk locally advanced squamous cell carcinoma of the head and neck. Cell Death Differ. 2024 Apr;31(4):460-68.

13. Heitzer E, Haque IS, Roberts CES, Speicher MR. Current and future perspectives of liquid biopsies in genomics-driven oncology. Nat Rev Genet. 2019 Feb;20(2):71-88.

14. Wilson HL, D'Agostino RB Jr, Meegalla N, Petro R, Commander S, Topaloglu U, et al. The Prognostic and Therapeutic Value of the Mutational Profile of Blood and Tumor Tissue in Head and Neck Squamous Cell Carcinoma. Oncologist. 2021 Feb;26(2):e279-e289.

15. Perdomo S, Avogbe PH, Foll M, Abedi-Ardekani B, Facciolla VL, Anantharaman D, et al. Circulating tumor DNA detection in head and neck cancer: evaluation of two different detection approaches. Oncotarget. 2017 Aug 7;8(42):72621-72632.

16. Galot R, van Marcke C, Helaers R, Mendola A, Goebbels RM, Caignet X, et al. Liquid biopsy for mutational profiling of locoregional recurrent and/or metastatic head and neck squamous cell carcinoma. Oral Oncol. 2020 May;104:104631.

17. Porter A, Natsuhara M, Daniels GA et al. Next generation sequencing of cell free circulating tumor DNA in blood samples of recurrent and metastatic head and neck cancer patients. Transl Cancer Res 2020; 9: 203-9.

18. Iams WT, Mackay M, Ben-Shachar R, Drews J, Manghnani K, Hockenberry AJ, et al. Concurrent Tissue and Circulating Tumor DNA Molecular Profiling to Detect Guideline-Based Targeted Mutations in a Multicancer Cohort. JAMA Netw Open. 2024 Jan 2;7(1):e2351700.

19. Nong J, Gong Y, Guan Y, Yi X, Yi Y, Chang L, et al. Circulating tumor DNA analysis depicts subclonal architecture and genomic evolution of small cell lung cancer. Nat Commun. 2018 Aug 6;9(1):3114.

20. Buttigieg MM, Rauh MJ. Clonal Hematopoiesis: Updates and Implications at the Solid Tumor-Immune Interface. JCO Precis Oncol. 2023 Jun;7:e2300132.

21. Pascual J, Gil-Gil M, Proszek P, Zielinski C, Reay A, Ruiz-Borrego M, et al. Baseline Mutations and ctDNA Dynamics as Prognostic and Predictive Factors in ER-Positive/HER2-Negative Metastatic Breast Cancer Patients. Clin Cancer Res. 2023 Oct 13;29(20):4166-77.

22. Roosan MR, Mambetsariev I, Pharaon R, Fricke J, Husain H, Reckamp KL, et al. Usefulness of Circulating Tumor DNA in Identifying Somatic Mutations and Tracking Tumor Evolution in Patients With Non-small Cell Lung Cancer. Chest. 2021 Sep;160(3):1095-07.

23. Liu Z, Xie Z, Zhao S, Ye D, Cai X, Cheng B, et al. Presence of allele frequency heterogeneity defined by ctDNA profiling predicts unfavorable overall survival of NSCLC. Transl Lung Cancer Res. 2019 Dec;8(6):1045-50.

24. Bidard FC, Kaklamani VG, Neven P, Streich G, Montero AJ, Forget F, et al. Elacestrant (oral selective estrogen receptor degrader) Versus Standard Endocrine Therapy for Estrogen Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: Results From the Randomized Phase III EMERALD Trial. J Clin Oncol. 2022 Oct 1;40(28):3246-56.

25. André F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, et al. Alpelisib for *PIK3CA*-Mutated, Hormone Receptor-Positive Advanced Breast Cancer. N Engl J Med. 2019 May 16;380(20):1929-40.