Received: 22 August 2022 | Revised: 7 November 2022 | Accepted: 2 February 2023

DOI: 10.1002/cac2.12410

CANCER COMMUNICATIONS Open Access

LETTER TO THE EDITOR

Integrated circulating tumor DNA and T cell repertoire predict radiotherapeutic response and outcome in non-small cell lung cancer patients with brain metastasis

Dear editor,

The scarcity of routine metastatic biopsies or resection limits the finding of biomarkers of diagnosis and prognosis in patients with brain metastases. Derived from necrosis, apoptosis, and secretion of tumor cells, circulating tumor DNA (ctDNA) is widely distributed in various body fluids, including peripheral blood and cerebrospinal fluid (CSF), as an alternative biomarker for tumor-associated analysis [1]. Fortunately, genomic alterations of blood ctDNA and CSF ctDNA have been proven as prognostic markers in non-small cell lung cancer (NSCLC) patients with brain metastasis [2, 3].

T cell-mediated immunity is critical in suppressing oncogenesis and metastasis of NSCLC. Targeted sequencing in the highly variable complementarity-determining region 3 (CDR3) region of the beta chain of the T cell repertoire (TCR) provides a robust method to quantify T cell diversity [4]. Casarrubios et al. [5] reported that the evenness of pretreatment TCR was related to the clinical response of NSCLC patients receiving neoadjuvant chemotherapy plus immunotherapy. Radiotherapy may stimulate immune responses against malignancies. Locally, irradiation causes antigen exposure by directly destroying cancer cells, which activates both the local and systemic immune systems. Irradiation can also cause DNA damage, and the ensuing mutations in cells with DNA mismatch repair deficiencies might increase the load of neoantigens, triggering an immunological response [6]. The PACIFIC trial demonstrated that combining radiotherapy and immunotherapy was effective in prolonging the survival of stage III NSCLC patients [7]. The TCR, which is closely associated with immune system modifications, also alters as a result of irradiation. Therefore, we investigated the dynamic changes of ctDNA and TCR during radiotherapy and the associations of ctDNA and TCR profiling in blood and CSF with radiotherapeutic responses and prognosis of NSCLC brain metastases.

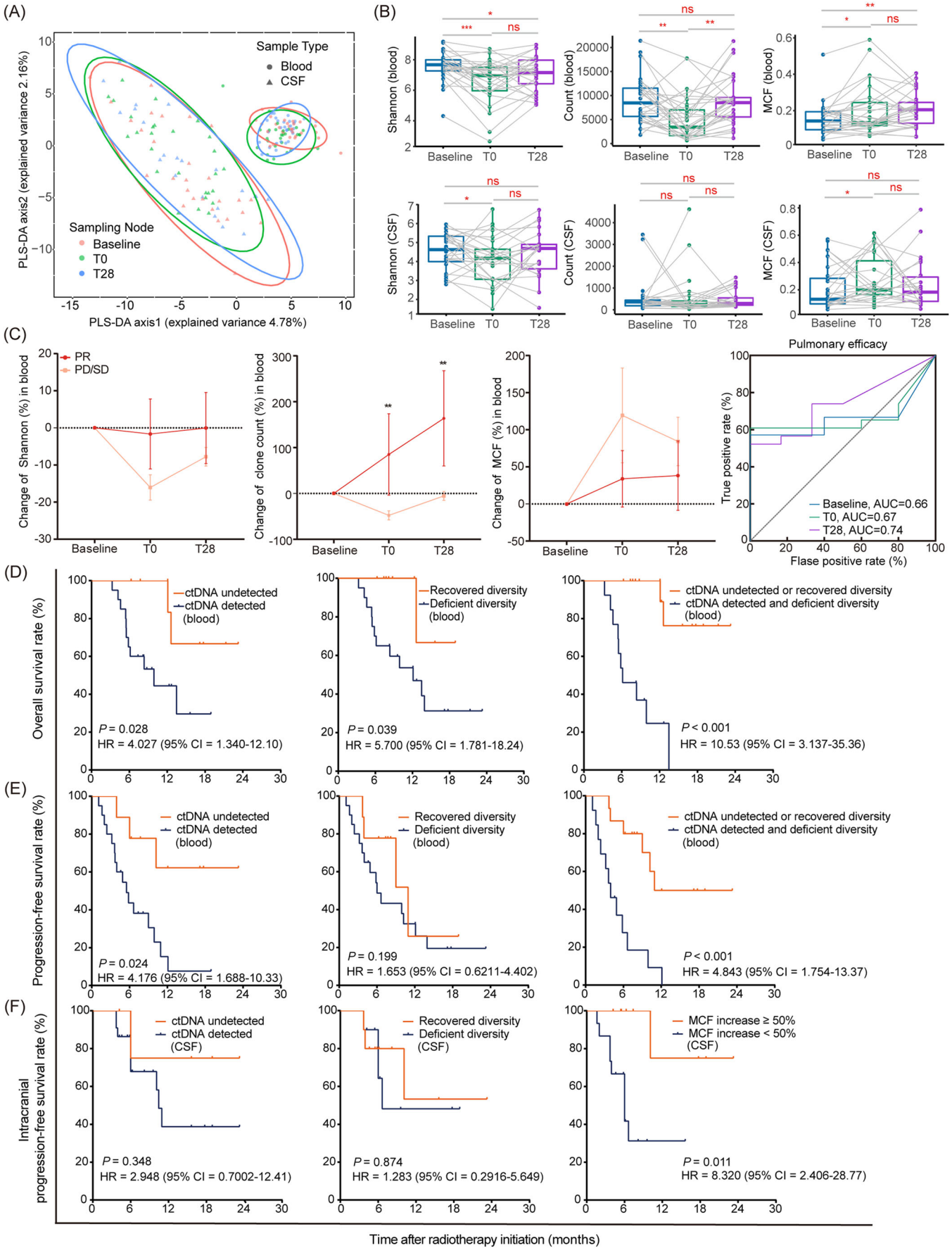
To address these questions, 30 NSCLC patients with brain metastases, diagnosed between February 2017 and December 2018, receiving brain radiotherapy were enrolled in the study (Supplementary Table S1). Peripheral blood and CSF samples were collected at baseline, 24 h (T0), and 28 days (T28) after completion of radiotherapy and underwent deep sequencing of ctDNA and TCR CDR3 regions (Supplementary Table S2). The 6-month response rates of brain metastases and systemic lesions were evaluated based on Response Evaluation Criteria in Solid Tumors version 1.1 for further study. Details of methods are provided in the Supplementary file.

Excluding samples with deficient cell free DNA (cfDNA) extraction, ctDNA was tested in 83 blood and 75 CSF samples (Supplementary Table S3). Collectively, 107 and 153 mutations, including single nucleotide variants and small insertions and deletions, were detected in 18 (69.2%) of 26 blood samples and 22 (81.5%) of 27 CSF samples at baseline, respectively. For 25 patients with paired blood-CSF samples with sufficient cfDNA extraction, 55 (27.4%) mutations were shared between blood and CSF samples in 9 patients, whereas 52 (25.9%) were detected only in blood samples and 94 (46.8%) only in CSF samples. The top ten mutation genes with the average variant allele frequency in blood and CSF were identical; however, the representative genes of various samples were distinct (Supplementary Figures S4-S5).

TCR sequencing was performed in 87 blood and 77 CSF samples (Supplementary Table S4). The results from partial least squares discriminant analysis showed that CSF TCR could be distinguished from the blood TCR

List of Abbreviations: cfDNA, Cell-free DNA; CSF, Cerebrospinal Fluid; ctDNA, Circulating Tumor DNA; HR, Hazard Ratio; iPFS, Intracranial Progression-free Survival; MAF, Maximum allele frequency; MCF, Maximum Clone Frequency; NSCLC, Non-small Cell Lung Cancer; OS, Overall Survival; PFS, Progression-free Survival; TCR, T cell Repertoire.

Ling Peng Yawen Bin Peng Ding contributed equally to this work.



via the dimensionality reduction of V and J gene usage (Figure 1A). The number of TCR clones was significantly greater in the blood than in the CSF at baseline, and few clones were shared (Supplementary Figure S6). These results suggested that the CSF and peripheral blood were independent compartments showing disparate genomic evolution and immune signatures.

Maximum allele frequency (MAF) and tumor mutational burden were the main parameters of ctDNA detection; shannon entropy, clone count, and the maximum clone frequency (MCF) were utilized to assess TCR diversity. The comparison of ctDNA and TCR key parameters at baseline revealed significant differences in MAF, Shannon entropy and clone number between blood and CSF (Supplementary Figure S7), supporting the previously stated view. Based on a pair-wise analysis, the Shannon entropy of both blood and CSF samples was decreased at T0 compared with baseline. The clone count in blood samples was significantly decreased at T0 compared with baseline, and then recovered at T28. However, the clone count in CSF samples showed no significant differences at the three time points. The MCF in both blood and CSF samples was increased at T0 compared with baseline, although the expansion pattern might be disparate. The MCF in blood samples at T28 was still greater than that at baseline, but there was no significant difference in MCF between baseline and T28 in CSF samples (Figure 1B).

The intracranial and systemic object response rates were 83.3% and 76.6% in our cohort, respectively. For patients with partial response, the dynamic change of blood Shannon entropy from baseline to T0 tended to be higher ($P = 0.097$ at T0, $P = 0.265$ at T28), and the clone count in each patient experienced a significant and persistent increase ($P = 0.007$ at T0, $P = 0.004$ at T28) compared with those with progressive disease or stable disease, indicating that patients with partial response seemed to maintain an activated immune repertoire to resist the radiotherapy-induced stress response. Receiver operating characteristic analysis further evaluated the predictive effect of allele frequency of blood ctDNA in distinguishing patients who would benefit from radiotherapy, and the area under curve reached 0.74 at T28 (Figure 1C).

With a median follow-up of 24 months, the overall survival (OS) was significantly increased in patients without ctDNA residual or with non-inferior Shannon entropy (defined as recovered diversity) in blood at T28. When we simultaneously looked at two factors, and the combination of ctDNA and TCR diversity exhibited an even better predictive performance for OS than individual ctDNA or TCR diversity [median: unreached vs. 6.1 months; hazard ratio (HR) = 10.53, $P < 0.001$] (Figure 1D). Similar results were obtained for progression-free survival (PFS) (Figure 1E).

Intracranial PFS (iPFS) was also regarded as an independent endpoint of this study. Patients with CSF MCF increased by ≥ 50% had longer iPFS than those with CSF MCF increased by <50% (median: unreached vs. 6.0 month; HR = 8.320, $P = 0.011$) (Figure 1F), indicating that the expansion of T cell clones in the CSF might serve as a potential prognostic marker for radiotherapy in the brain metastasis treatment. In addition, the combination of ctDNA and CSF MCF did not enhance the predictive power (Supplementary Figure S8).

The combination of ctDNA residual and TCR diversity change in blood at T28 was still powerful in predicting OS (HR = 0.024, $P < 0.001$) and PFS (HR = 0.083, $P < 0.001$) independent of clinical factors in multivariate Cox model analysis. Especially, the MCF with ≥ 50% increase in CSF was also an independent protective factor for iPFS (HR = 0.028, $P = 0.008$) (Supplementary Table S5). Note that systemic treatment may bias research results; this effect needs to be validated in a larger sample size.

This study characterized the longitudinal profile of genomic and immune statuses in both CSF and peripheral blood samples from NSCLC patients with brain metastases treated with cranial radiotherapy. TCR in blood boosted the prognostic capacity of ctDNA in NSCLC patients following brain radiotherapy, whereas TCR in CSF could predict the iPFS independently.

FIGURE 1 Therapeutic efficacy associated with TCR dynamic and ctDNA burden in peripheral blood and CSF throughout radiotherapy for brain metastasis in non-small cell lung cancer patients. (**A**) PLS-DA clusters reveal distinct V and J gene recombination of blood (circle) and CSF (triangle) TCR. All samples with available TCR results were included. (**B**) Broken line graphs showing the dynamic changes of Shannon entropy, clone count, and MCF in blood or CSF sample from each patient. Statistical analysis was performed using paired-sample *t*-tests (two-sided). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, no statistical significance. (**C**) The left three panels indicate the percentage changes of blood TCR parameters in patients with different pulmonary responses, and the right panel indicates the differential prediction capability of ctDNA AF in baseline, T0, and T28 blood samples using ROC analysis. (**D-F**) Kaplan-Meier curves are shown for OS (**D**), PFS (**E**), and iPFS (**F**). The blood or CSF ctDNA and TCR parameters were tested. ctDNA status was determined at T28, and recovered diversity indicates non-inferiority Shannon at T28 compared with baseline. The greater MCF increase at T0 or T28 benchmarked baseline is identified as the basis for grouping.

Abbreviations: T0, within 24 h after completion of radiotherapy; T28, 28 days after completion of radiotherapy; AF, allele frequency; AUC, area under curve; CSF, cerebrospinal fluid; ctDNA, circulating tumor DNA; HR, hazard ratio; iPFS, intracranial PFS; MCF, maximum clone frequency; OS, overall survival; PFS, progression-free survival; PLS−DA, partial least squares discriminant analysis; ROC, receiver operating characteristic; RT, radiotherapy; SRT, stereotactic radiotherapy; TCR, T cell repertoire; WBRT, whole-brain radiotherapy.

DECLARATIONS

AUTHOR CONTRIBUTIONS

Ling Peng: Conceptualization, Methodology. Yawen Bin: Investigation, Data curation. Peng Ding and Lingjuan Chen: Formal analysis, Writing-Original draft preparation. Hao Zeng, Zelong Xu, Liyan Ji and Xuan Gao: Formal analysis, Visualization and Sequencing. Ye Wang and Sheng Zhang: Radiotherapy technical support. Pian Liu and Zhongxing Liao: Clinical research support, Formal analysis. Xuefeng Xia and Ruiguang Zhang: Project administration, Validation. Fan Tong and Xiaorong Dong: Writing- Reviewing and Editing, Supervision and Funding acquisition.

ACKNOWLEDGMENTS

The authors would like to thank the patients, their families and caregivers, trial nurses, data managers, and all study investigators for their contributions to the study.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted according to the Declaration of Helsinki. All patients provided written informed consent before study procedures began. The protocol was approved by the Institutional Review Board of Union Hospital affiliated to Tongji Medical College, Huazhong University of Science and Technology (No. 0116) and registered on the ClinicalTrials.gov under the number NCT05737589. This work was supported by the National Natural Science Foundation of China (81573090, 81773233).

CONSENT FOR PUBLICATION

Written informed consent for publication was obtained from all participants.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article and its supplementary information files or from the corresponding author upon reasonable request.

Ling Peng[1]
Yawen Bin[1]
Peng Ding[1]
Lingjuan Chen[1]
Hao Zeng[1]
Zelong Xu[2]
Liyan Ji[2]
Xuan Gao[3,4]
Pian Liu[1]
Ye Wang[1]
Sheng Zhang[1]
Zhongxing Liao[5]
Xuefeng Xia[2]
Ruiguang Zhang[1]
Fan Tong[1]
Xiaorong Dong[1]

[1]*Cancer Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, P. R. China*
[2]*Geneplus-Beijing, Beijing, P. R. China*
[3]*State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing, P. R. China*
[4]*GenePlus-Shenzhen Clinical Laboratory, Shenzhen, Guangdong, P. R. China*
[5]*Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA*

Correspondence
Dr. Xiaorong Dong, Fan Tong, and Ruiguang Zhang, Cancer Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China.
Email: xiaorongdong@hust.edu.cn, tongfan.1986@163.com, zrg27@163.com

ORCID

Liyan Ji https://orcid.org/0000-0001-5680-9579
Xiaorong Dong https://orcid.org/0000-0001-7470-1836

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.