Clinical value of alveolar lavage supernatant specimens in the detection of the EGFR gene mutation in patients with non-small cell lung carcinoma

Songyan Han1#, Yanrong Guo1#, Xinwei Luo2#, Guoping Tong1, Chang Zhao1, Yuan Li1, Tingting Guo1, Li Wang1, Ning Gao3, Yuexiang Liu3, Hongwei Li1, Weihua Yang1

1First Respiratory Department, Shanxi Province Cancer Hospital/Shanxi Hospital Affiliated to Cancer Hospital, Chinese Academy of Medical Sciences/Cancer Hospital Affiliated to Shanxi Medical University, Tai'yuan, China; 2Department of Respiratory and Critical Care Medicine, Chongqing University Three Gorges Hospital, Chongqing, China; 3Department of Pathology, Shanxi Province Cancer Hospital/Shanxi Hospital Affiliated to Cancer Hospital, Chinese Academy of Medical Sciences/Cancer Hospital Affiliated to Shanxi Medical University, Tai'yuan, China; 4Department of Thoracic Radiotherapy, Shanxi Province Cancer Hospital/Shanxi Hospital Affiliated to Cancer Hospital, Chinese Academy of Medical Sciences/Cancer Hospital Affiliated to Shanxi Medical University, Tai'yuan, China

Contributions: (I) Conception and design: S Han, W Yang, Y Guo; (II) Administrative support: W Yang, H Li; (III) Provision of study materials or patients: Y Guo, X Luo, G Tong, C Zhao, Y Li; (IV) Collection and assembly of data: T Guo, L Wang; (V) Data analysis and interpretation: S Han, W Yang, H Li, Y Guo, N Gao, Y Liu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

These authors contributed equally to this work.

Background: This study sought to compare the consistency of the epidermal growth factor receptor (EGFR) gene mutation detection results in the supernatant of alveolar lavage specimens to the tissue sample results, and the consistency of the blood EGFR gene mutation detection results to the tissue detection results.

Methods: In total, 29 patients with non-small cell lung carcinoma (NSCLC) were selected, and their bronchoalveolar lavage fluid (BALF) was collected. The supernatant and precipitate were separated by centrifugation. Deoxyribonucleic acid (DNA) was extracted from the supernatant, and blood and tumor tissues were collected to detect patients’ EGFR gene mutation status.

Results: Of the 29 enrolled patients, 12 of the 23 tissue-biopsy patients (52.2%) were positive for EGFR mutations, 11 of the 28 blood-test patients (39.2%) were positive for EGFR mutations, and 13 of the 29 cases of the BALF-test patients (44.8%) were positive for EGFR mutations. The most common mutations were the exon 19 deletion mutation and the L858R point mutation. The EGFR gene mutation rate was higher in female, young, non-smoker, and stage IIIB patients (than stage IV patients), but the differences were not statistically significant (all P>0.05). Of the 29 NSCLC patients tested for the EGFR gene mutation, the BALF supernatant and blood results were the same for 27 patients (coincidence rate: 93.10%). Of the 23 of the 29 enrolled patients tested for the EGFR gene mutation, the BALF supernatant and tissue test results were the same for 21 patients (coincidence rate: 91.30%). Further, the blood-test and the tissue test results were the same for 20 patients (coincidence rate:86.96%).

Conclusions: The EGFR gene mutation rate was high in NSCLC patients. The coincidence rate of the EGFR gene mutation detection results between BALF supernatant and tumor tissues was slightly higher than that of the blood and tumor tissue EGFR gene mutation detection results.

Keywords: Bronchoalveolar lavage fluid (BALF); non-small cell lung carcinoma (NSCLC); epidermal growth factor receptor (EGFR)

Submitted Jan 27, 2022. Accepted for publication May 13, 2022.
doi: 10.21037/tcr-22-681

View this article at: https://dx.doi.org/10.21037/tcr-22-681
Introduction

Lung carcinoma is the most common malignant tumor. Advanced non-small cell lung carcinoma (NSCLC) is the most common type of lung carcinoma, and has a high mortality rate and poor prognosis (1). The epidermal growth factor receptor (EGFR) is a tyrosine kinase type receptor, which leads to receptor dimerization, tyrosine kinase activation, the phosphorylation of specific tyrosine residues, and ultimately, also activates the mitogen-activated protein kinase, protein kinase B, and c-Jun N-terminal kinase, signaling pathways, leading to deoxyribonucleic acid (DNA) synthesis, cell proliferation, and differentiation (2).

The overexpression, abnormal expression, and mutation of the EGFR gene are closely related to NSCLC. The frequency of EGFR mutation in China, is higher than that in Europe, which is about 50% in China (3), and about 15% in Europe (4). The most common EGFR gene mutations in NSCLC patients are exon 19 deletion (19del) and exon 21 arginine substitution leucine (L858R) point mutations, which account for 90% of the EGFR gene mutations in NSCLC patients (5). Through the competitive binding of EGFR, tyrosine kinase inhibitors (TKIs) inhibit the EGFR signaling pathway, promote apoptosis, inhibit tumor cell proliferation and metastasis, prolong the survival time of patients, and improve their quality of life (6). Thus, it is of great clinical significance to detect the mutation status of the EGFR gene in patients with NSCLC, which is a prerequisite for determining whether patients can undergo targeted therapy.

Chemotherapy is the traditional treatment for NSCLC patients with positive EGFR gene mutation detection results, but it has severe side effects. Large clinical trials at home and abroad have compared the efficacy of chemotherapy and TKI-targeted therapy in NSCLC patients with positive tissue EGFR gene mutation detection results, and found that after TKI-targeted therapy, advanced patients with EGFR mutation positive results have prolonged progression free survival and an improved objective response rate (7,8). Consequently, the detection of the EGFR gene mutation before NSCLC treatment has gradually become routine.

The detection of EGFR mutations in tumor tissues is the gold-standard method for determining whether patients are likely to benefit from EGFR TKI-targeted therapy. The clinical application of tissue, which is the main material for detecting the EGFR gene mutation in NSCLC patients, is limited by various factors, such as the limitation of advanced stage and insufficient source of tumor tissue, the heterogeneity of the EGFR gene mutation, and the long detection cycle. Additionally, for NSCLC patients with multiple metastases undergoing TKI-targeted therapy, it is necessary to detect early evidence of recurrence or find the mechanism of potential drug resistance. Tissue biopsies are expensive. Conversely, peripheral blood detection is a simple, rapid and practical method with high specificity that overcomes tumor heterogeneity to a certain extent (9). Additionally, the consistency of tissue detection results can reach >80% (10). Blood sample plays a supplementary role in tissue detection and represents an important development direction with clinical application in the future.

In 2015, China’s State Food and Drug Administration proposed that if the EGFR gene status of tumor samples could not be evaluated, circulating tumor DNA (ctDNA) obtained from blood (plasma) could be used for the evaluations (11). However, most of the techniques for detecting plasma EGFR gene mutations are not sensitive, and there are differences in sensitivity among tumor patients at different stages. Additionally, the sensitivity of the detection technique needs to be high (12), as the proportion of ctDNA adopted for EGFR gene detection in plasma cell-free DNA (cfDNA) is <1%.

The cytological examination of bronchoalveolar lavage fluid (BALF) has been widely adopted in the screening and diagnosis of lung tumors. However, the supernatant of BALF has not yet been used to detect the EGFR gene mutation in patients with NSCLC. For advanced patients, it is difficult to get the tissue sample, however because of the character of cancer rapid evolution during the treatment, we can’t use tissue samples from a few years ago. BALF analysis may replace cancer tissue examination, which is of limited access in advanced stages. Though study has showed that BALF can be used to detect EGFR mutation and guide target therapy, but we do not get enough information about its sensitivity and specify, and if it’s better than the tissue and plasma (13). This study sought to explore the feasibility and application value of the BALF supernatant in the diagnosis of the EGFR gene mutation in NSCLC patients by means of polymerase chain reaction detection to determine if it could be adopted as a supplement or substitute for tissue detection to improve the overall detection rate of the EGFR gene and the positive detection rate of the EGFR gene mutation in NSCLC patients.

This study also sought to provide more evidence for the clinical guidance of the individualized treatment of NSCLC. We present the following article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-681/rc).
Methods

General data
In total, 29 patients with NSCLC diagnosed at the Shanxi Province Cancer Hospital from March 2016 to May 2019 were enrolled in this study. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study has been approved by the ethics committee of Chinese Academy of Medical Sciences/Cancer Hospital Affiliated to Shanxi Medical University (No. 201636). All patients have signed informed consent to enroll in this study. To be eligible for inclusion in this study, patients had to meet the following inclusion criteria: (I) be newly diagnosed with NSCLC; or (II) have recurrent NSCLC. Patients were excluded from the study if they met any of the following exclusion criteria: (I) the pathological type of lung carcinoma could not be identified; and/or (II) the NSCLC patients could not tolerate a fiberoptic bronchoscopy.

Based on the inclusion and exclusion criteria, a total of 29 patients with NSCLC were included in the study. Of the 29 patients, 18 were male (62.1%) and 11 were female (37.9%). The patients had a mean age of 63.0 (range: 47.8–67.0) years, but the ages of 3 patients were unknown. Among the patients, 11 had a history of smoking (64.7%), but 16 had an unknown smoking history, and 9 had clinical stage III, 8 had stage IV, but the clinical stage of 12 was unknown.

Main reagents and instruments
The nucleic acid extraction reagents (circulating DNA), human EGFR gene mutation detection reagents (Super-ARMS method), nucleic acid extraction reagents (tissue, pleural effusion DNA), and human EGFR gene mutation detection reagents (ADxARMS®) were all purchased from Xiamen Biopharmaceutical Technology Co. Ltd.

Research methods
Sample collection and DNA extraction of BALF
The location of each lesion was determined by chest imaging examination. The site with the most significant lesion or the site with the fastest progression was selected for lavage. Lidocaine (2%; 1–2 mL) was injected into the lavaged lung segment through the biopsy hole, and local anesthesia was performed in the lavaged lung segment. At 37 °C or room temperature, aseptic saline was quickly injected through the operating channel, and after the top of the bronchoscope had been embedded in the target bronchial segment or sub-end opening, the BALF was immediately obtained by negative pressure suction. On the day on which the BALF samples were collected, 30 mL of BALF was taken into the centrifuge tube, and the supernatant and precipitate were separated by 2-stage centrifugation (i.e., 2,000 ×g centrifugation for 10 min, and 8,000 ×g centrifugation of the supernatant for 10 min). The supernatant was transferred to a test tube, and cfDNA in the supernatant was extracted using an Ed biological cfDNA extraction kit, and stored in a refrigerator at 20 °C. The remaining precipitated DNA in BALF after centrifugation was extracted using a biological tissue and pleural effusion DNA separation kit, and stored in a refrigerator at –20 °C.

Collection of blood samples (9,11) and extraction of DNA
Whole blood (10 mL) was collected during the morning following hospitalization. A 2-stage centrifugation method was adopted (centrifugation for 10 min at 800 ×g, centrifugation for 5 min at 16,000 ×g, transference of the supernatant to the test tube), and the sample was isolated for <2 hours. The sample size was about 4–5 mL. A cfDNA extraction kit was used to extract the cfDNA from the plasma.

Collection of tissue specimens and extraction of DNA
The tumor tissue samples were obtained by endobronchial ultrasound-guided fine-needle biopsy, fiberoptic bronchoscopic biopsy, and percutaneous lung biopsy. The DNA was extracted from the tissue samples using a biological tissue and pleural effusion DNA separation kit.

EGFR test after DNA extraction
The EGFR in the BALF supernatant and the EGFR in the blood were detected using the Super-ARMS (amplification refractory mutation system, ARMS) method, and the EGFR in the tumor tissue was detected by ADx-ARMS®. Additionally, the mutation of the EGFR gene was detected according to the operating procedures of the instructions, and the results were interpreted according to the instructions.

Statistical analysis
All the data were statistically analyzed using SPSS25.0 software. The measurement data with a normal distribution
and uniform variance are expressed as the x ± s, and the t-test was used for comparisons between the groups. The data with a non-normal distribution are expressed as the median (first quartile, third quartile). The counting data are expressed as the case and rate (%). For the comparisons between groups, Fisher’s exact probability method was used. A P value <0.05 indicated a statistically significant difference.

Results

Results of EGFR gene mutation detection

Of the 23 patients who underwent tissue examinations, 12 (52.2%) were positive for the EGFR gene mutation, 8 (66.7%) for the 19del mutation, and 4 (33.3%) for the L858R mutation. Additionally, of the 28 patients who had blood tests, 11 (39.2%) were positive for the EGFR gene mutation, 7 (63.6%) for the 19del mutation, and 4 (36.4%) for the L858R mutation. Further, of the 29 patients who underwent BALF supernatant examinations, 13 (44.8%) were positive for the EGFR gene mutation, 8 for the (61.5%) 19del mutation, and 5 (38.5%) for the L858R mutation.

The results of the EGFR gene mutation detection in the different samples were consistent

Of the 29 NSCLC patients tested for the EGFR gene mutation, the BALF supernatant and blood-test results were the same for 27 patients (coincidence rate: 93.10%). Of the 23 of the 29 patients tested for the EGFR gene mutation, the BALF supernatant and tissue test results were the same for 21 patients (coincidence rate: 91.30%). Further, the blood-test and the tissue test results were the same for 20 patients (coincidence rate: 86.96%). The coincidence rate for the BALF supernatant and the tissue EGFR gene status was slightly higher than that of the blood and tissue EGFR gene status.

Relationship between EGFR gene mutation and clinicopathological features

Among the 23 patients who underwent tissue examination, 8 of the patients were female, 6 of whom had the EGFR gene mutation (75.0%), and 15 were male, 6 of whom had the EGFR gene mutation (40.0%). The mutation rate in females was higher than that in males, but there was no significant difference between the 2 groups (P>0.05). Among the 10 patients aged >60 years, 5 (50.0%) were positive for the EGFR gene mutation, and among the 11 patients aged <60 years, 5 (45.5%) were positive for the EGFR gene mutation, but the difference between the 2 groups was not statistically significant (P>0.05). Among the 5 non-smoking patients, 4 (80.0%) had the EGFR gene mutation, and among the 9 smoking patients, 4 (44.4%) had the EGFR gene mutation. The mutation rate of the non-smoking patients was higher than that of the smoking patients, but the difference was not statistically significant (P>0.05). Among the 9 patients with clinical stage IIIIB, 5 (55.6%) were positive for the EGFR gene mutation, and among the 6 patients with clinical stage IV, 3 (50.0%) were positive for the EGFR gene mutation, but the difference was not statistically significant (P>0.05; see Table 1).

Discussion

TKI-targeted therapy based on EGFR gene detection has brought accuracy to the medical treatment of lung carcinoma. And now many target drugs like osimertinib, erlotinib, afatinib, gefitinib, dacomitinib have approved by FDA, and osimertinib as the first line used for patients with EGER mutation, the OS has achieved 33.1 months in China (14). Consequently, understanding the status of the EGFR mutation is of great significance to clinical decision making for patients with advanced NSCLC (especially lung adenocarcinoma) (15).

This study showed that the coincidence rate between the supernatant of the BALF and tissue EGFR gene status (91.3%) was slightly higher than that of the blood and tissue EGFR gene status (86.96%). EGFR gene mutation detection has become a key measure for the clinical treatment and efficacy evaluation of NSCLC patients. Tissue detection, which is the gold standard for determining the EGFR gene status, is limited by various factors in clinical practice, and its actual application rate is low. It is necessary to seek other biological samples that can be adopted for EGFR gene status detection to make up for the deficiency of tissue detection. BALF obtained by fiberoptic bronchoscope has been adopted as a new biological sample for EGFR detection. Compared to tissue detection, the detection results have high consistency, and have great potential for development. However, it is still necessary to overcome the problem of the low sensitivity of the detection and analysis techniques for the detection of the EGFR gene in non-tissue samples.

The common EGFR mutations are more likely to be associated with female patients or never-smokers, but a few
Table 1 Relationship between EGFR gene mutation and clinicopathological features in patients with NSCLC

| Clinical features         | Total | Detection results of tissue EGFR gene | P    |
|---------------------------|-------|--------------------------------------|------|
|                           |       | Mutation positive | Mutation negative |      |
| Gender                    |       |                      |                  |      |
| Male                      | 15    | 6 (40.0%)             | 9 (60.0%)        | 0.193|
| Female                    | 8     | 6 (75.0%)             | 2 (25.0%)        |      |
| Age* (years)              |       |                      |                  | 1.000|
| >60                       | 10    | 5 (50.0%)             | 5 (50.0%)        |      |
| ≤60                       | 11    | 5 (45.5%)             | 6 (54.5%)        |      |
| Smoking^b                 |       |                      |                  | 0.301|
| No                        | 5     | 4 (80.0%)             | 1 (20.0%)        |      |
| Yes                       | 9     | 4 (44.4%)             | 5 (55.6%)        |      |
| Clinical staging^c        |       |                      |                  | 1.000|
| IIIB                      | 9     | 5 (55.6%)             | 4 (44.4%)        |      |
| IV                        | 6     | 3 (50.0%)             | 3 (50.0%)        |      |

Of the 23 patients who underwent tissue examinations, 12 (52.2%) were positive for the EGFR gene mutation, 8 (66.7%) for the 19del mutation, and 4 (33.3%) for the L858R mutation. *, except for 21 patients with exact age information, the age of the other 2 patients is unknown; ^b, except for 14 patients with smoking, smoking history of the other 9 patients is unknown; ^c, except for 15 patients with definite clinical stage, 8 patients with unknown clinical stage.

rare mutations, like exon 20 mutations, are not associated with any clinicopathological features (16). This study showed that the gene mutation rate of female patients with NSCLC was higher than that of male patients, and the gene mutation rate of elderly patients was higher than that of younger patients. Additionally, the gene mutation rate of non-smoking patients was higher than that of smoking patients, and the gene mutation rate of stage IIIB patients was higher than that of stage IV patients, but the difference was not statistically significant (P>0.05). The results of this study differ to those of previous study (15). This may be because (I) this study is an observational study and had fewer patients than the multicenter prospective studies; (II) the results of the study may have been affected by underreporting, as some patients concealed their smoking history; and (III) the air pollution in Shanxi Province, which is serious due to its geographical location and industrial production, led to an increase in the incidence of the inhalation of harmful substances in patients with no history of smoking. However, both previous studies and our studies suggest that more attention should be paid to the status of the EGFR gene in women and patients with no history of smoking.

Conclusions

The detection of the EGFR gene mutation is a key prerequisite for TKI-targeted therapy among NSCLC patients. The selection of suitable non-tissue biological samples for detection can improve the overall EGFR gene detection rate and the positive detection rate of EGFR gene mutations in NSCLC patients; thus, promoting the progress of individualized therapy in NSCLC patients. The coincidence rate of the EGFR gene mutation detection in the BALF supernatant and tissue EGFR gene mutation detection was slightly higher than that of the plasma and tissue EGFR gene mutation detection, which makes BALF a better genetic testing sample. However, to ensure more NSCLC patients can benefit from TKI-targeted therapy, further research needs to be conducted to improve the detection sensitivity and analysis technology, and standardize the relevant clinical operation. Currently, there is no standard method and operation flow for detecting the EGFR gene mutation in BALF in clinical practice.
Acknowledgments

Funding: None.

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-681/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-681/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-681/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study has been approved by the ethics committee of Chinese Academy of Medical Sciences/Cancer Hospital Affiliated to Shanxi Medical University (No. 201636). All patients have signed informed consent to enroll in this study.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: https://creativecommons.org/licenses/by-nc-nd/4.0/.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global carcinoma statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 carcinomas in 185 countries. CA Cancer J Clin 2018;68:394-424.

2. Morgan BM, Lamisa Q, Matthew V, et al. Biochemistry, Epidermal Growth Factor Receptor. Treasure Island (FL): StatPearls, 2022.

3. Shi Y, Au JS, Thongprasert S, et al. A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIioneer). J Thorac Oncol 2014;9:154-62.

4. Kerr KM, Bibeau F, Thunnissen E, et al. The evolving landscape of biomarker testing for non-small cell lung cancer in Europe. Lung Cancer 2021;154:161-75.

5. Roskoski R Jr. The ErbB/HER family of protein-tyrosine kinases and carcinoma. Pharmacol Res 2014;79:34-74.

6. Yoneda K, Imanishi N, Ichiki Y, et al. Treatment of Non-Small Cell Lung Cancer with EGFR-mutations. J UOEH 2019;41:153-63.

7. Wu YL, Saijo N, Thongprasert S, et al. Efficacy according to blind independent central review: Post-hoc analyses from the phase III, randomized, multicenter, IPASS study of first-line gefitinib versus carboplatin/paclitaxel in Asian patients with EGFR mutation-positive advanced NSCLC. Lung Cancer 2017;104:119-25.

8. Zhou C, Wu YL, Chen G, et al. Final overall survival results from a randomised, phase III study of erlotinib versus chemotherapy as first-line treatment of EGFR mutation-positive advanced non-small-cell lung cancer (OPTIMAL, CTONG-0802). Ann Oncol 2015;26:1877-83.

9. Wang Z, Cheng Y, An T, et al. Detection of EGFR mutations in plasma circulating tumour DNA as a selection criterion for first-line gefitinib treatment in patients with advanced lung adenocarcinoma (BENEFIT): a phase 2, single-arm, multicentre clinical trial. Lancet Respir Med 2018;6:681-90.

10. Xie F, Zhang Y, Mao X, et al. Comparison of genetic profiles among primary lung tumor, metastatic lymph nodes and circulating tumor DNA in treatment-naive advanced non-squamous non-small cell lung cancer patients. Lung Cancer 2018;121:54-60.

11. Swinkels DW, Wiegerinck E, Steegers EA, et al. Effects of blood-processing protocols on cell-free DNA quantification in plasma. Clin Chem 2003;49:525-6.

12. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014;6:224ra24.

13. Lee SE, Park HY, Hur JY, et al. Genomic profiling of extracellular vesicle-derived DNA from bronchoalveolar lavage fluid of patients with lung adenocarcinoma. Transl Lung Cancer Res 2021;10:104-16.

14. Cheng Y, He Y, Li W, et al. Osimertinib Versus Comparator EGFR TKI as First-Line Treatment for EGFR-Mutated Advanced NSCLC: FLAURA China, A
Randomized Study. Target Oncol 2021;16:165-76.

15. Shi Y, Li J, Zhang S, et al. Molecular Epidemiology of EGFR Mutations in Asian Patients with Advanced Non-Small-Cell Lung Cancer of Adenocarcinoma Histology - Mainland China Subset Analysis of the PIONEER study. PLoS One 2015;10:e0143515.

16. Wei B, Ren P, Zhang C, et al. Characterization of common and rare mutations in EGFR and associated clinicopathological features in a large population of Chinese patients with lung cancer. Pathol Res Pract 2017;213:749-58.

Cite this article as: Han S, Guo Y, Luo X, Tong G, Zhao C, Li Y, Guo T, Wang L, Gao N, Liu Y, Li H, Yang W. Clinical value of alveolar lavage supernatant specimens in the detection of the EGFR gene mutation in patients with non-small cell lung carcinoma. Transl Cancer Res 2022;11(5):1188-1194. doi: 10.21037/tcr-22-681